Patents Act 1977 Examiner's report to the Comptroller under Section 17 The Search report) 48	Application number GB 9400882.8
Relevant Technical Fields (i) UK Cl (Ed.M) (ii) Int Cl (Ed.5) C07J C07D C07C	Search Examiner R HONEYWOOD Date of completion of Search 19 APRIL 1994
Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE DATABASE - CAS	Documents considered relevant following a search in respect of Claims :- 1-10

Categories of documents

X:	Document indicating lack of novelty or of inventive step.	P:	Document published on or after the declared priority date but before the filing date of the present application.
Y:	Document indicating lack of inventive step if combined with one or more other documents of the same category.	E:	Patent document published on or after, but with priority date earlier than, the filing date of the present application.
A:	Document indicating technological background and/or state of the art.	&:	Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	GB 760346	(ROCHE PRODUCTS LTD)	1 at least
X	EP 0195311 A2	(YOSHIKAWA OIL AND FAT CO LTD) see example 49 run 19	1 at least
X	US 5198432 A	(CENTER FOR INNOVATIVE TECHNOLOGY)	1 at least
X	CH 681891 A	(MARIGEN)	1 at least
X	CA 108 (22): 194660g L STUD ORG CHEM (AMSTERDAM), 30 (RECENT ADV ELECTROORG SYNTH 113-16 (see abstract)		1 at least
X	CA 97(14): 117240x 2H FIZ KHIM, 56(6) 1515-6 (see abstract)		1 at least

Databases:The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).

(12) UK Patent Application (19) GB (11) 2 285 805 (13) A

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C2U U4A1B U4C1 U4C2 U6B U6C
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U1S S1313

(56) Documents Cited
GB 0760346 A EP 0195311 A2 CH 000681891 A
US 5198432 A
CA 108(22):194660 g CA 97(14):117240 x

(58) Field of Search
INT CL[5] C07C , C07D , C07J
ONLINE DATABASE - CAS

(54) Esters

(57) New esters possessing antitumor activity of saturated and unsaturated dicarbonic acids with sterols, vitamin-D and vitamin-E compounds, processes for their production, as well as for the preparation of spontaneously dispersible concentrates and pharmaceutical compositions containing these esters with dicarbonic acids are described.

GB 2 285 805 A

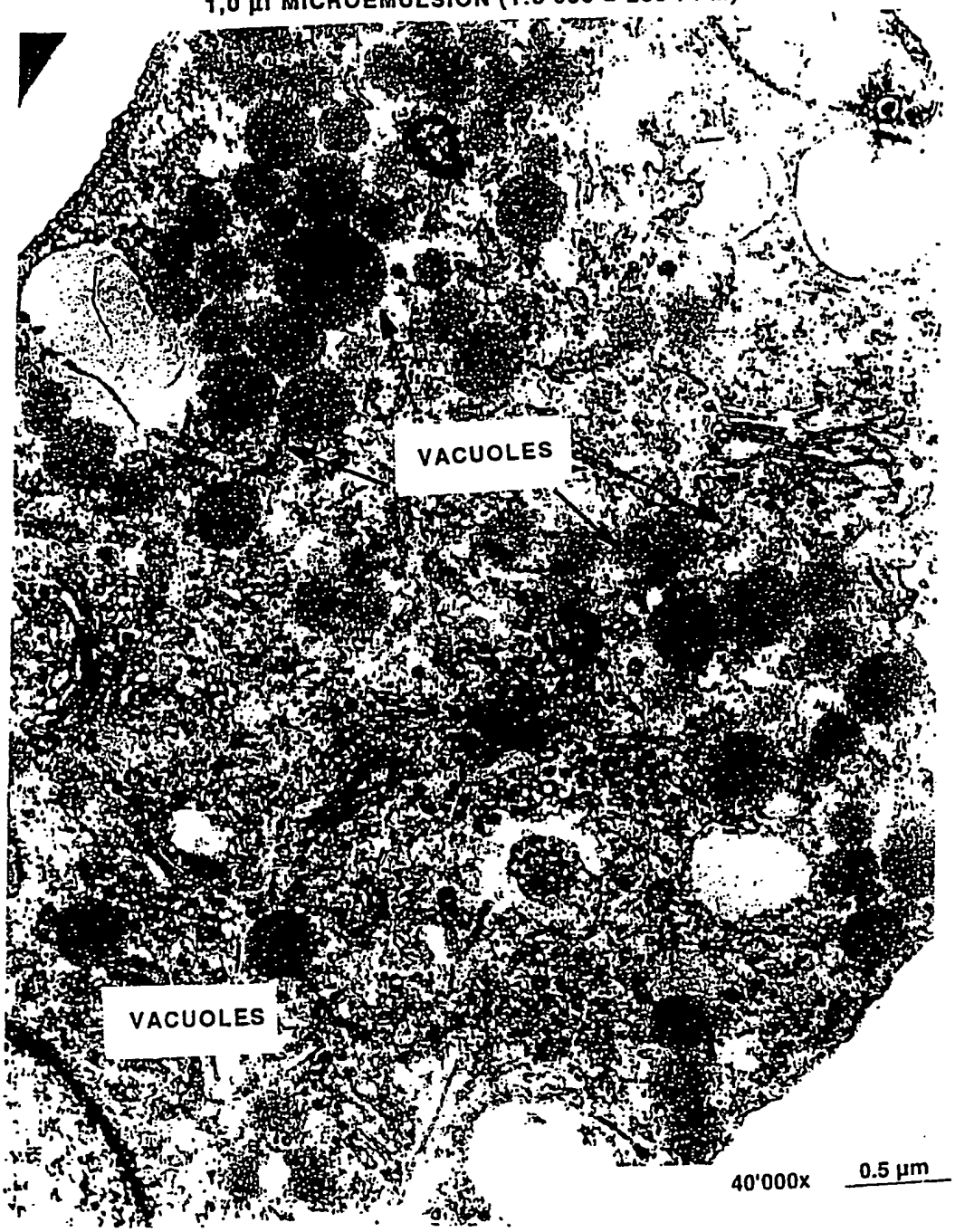
At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

PENETRATION at the TUMOUR CELL MEMBRANE
2 %-MARIGENOL®-CONCENTRATE
with ERGOSTERYL-10-UNDECENOATE

FIGURE 1

PY6 CELLS

1,0 µl MICROEMULSION (1:5'000 = 200 PPM)

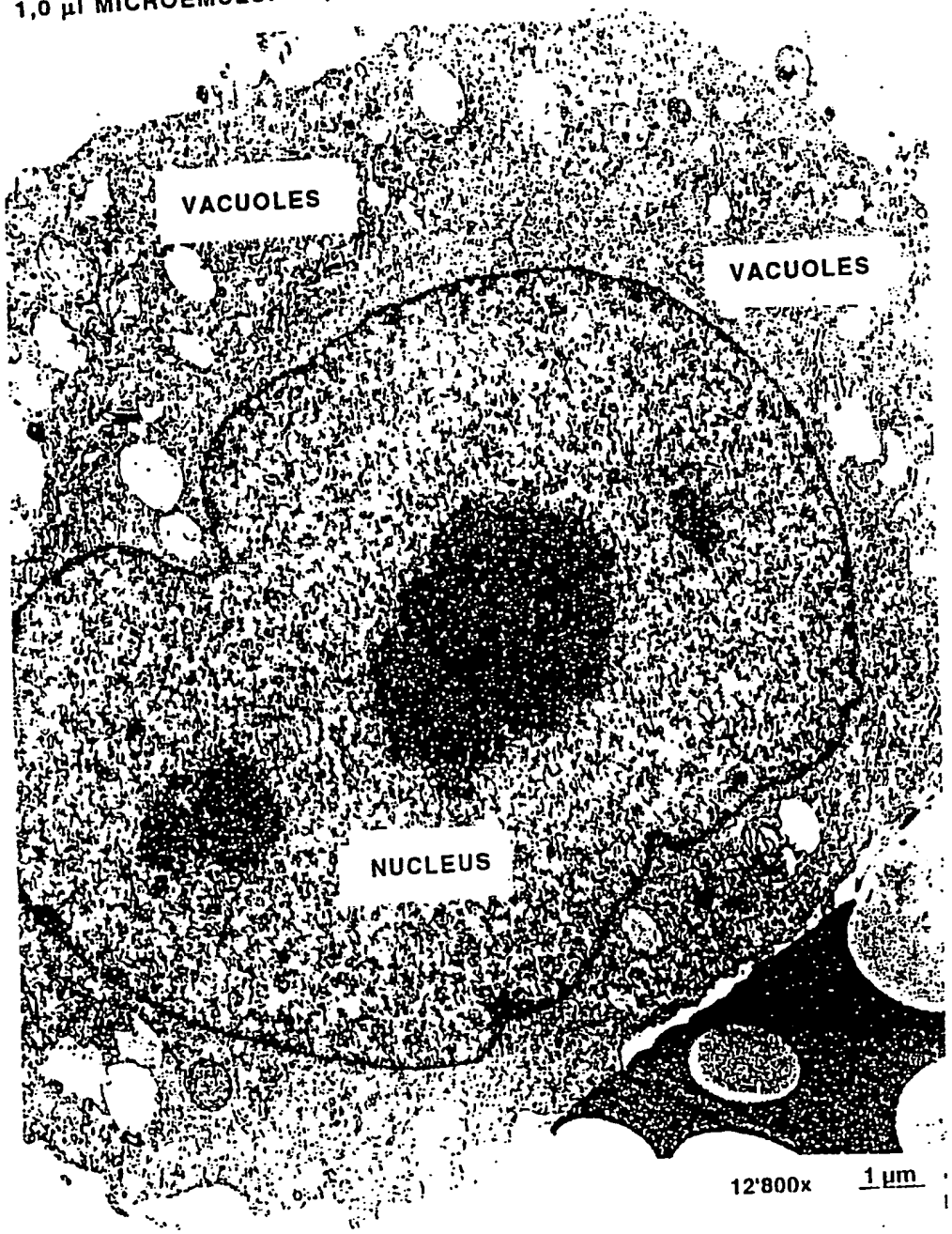


PENETRATION at the TUMOUR CELL MEMBRANE
2 %-MARIGENOL®-CONCENTRATE
with ERGOSTERYL-all trans-RETINATE

PY6 CELLS

FIGURE 2

1,0 µl MICROEMULSION (1:5'000 = 200 PPM)



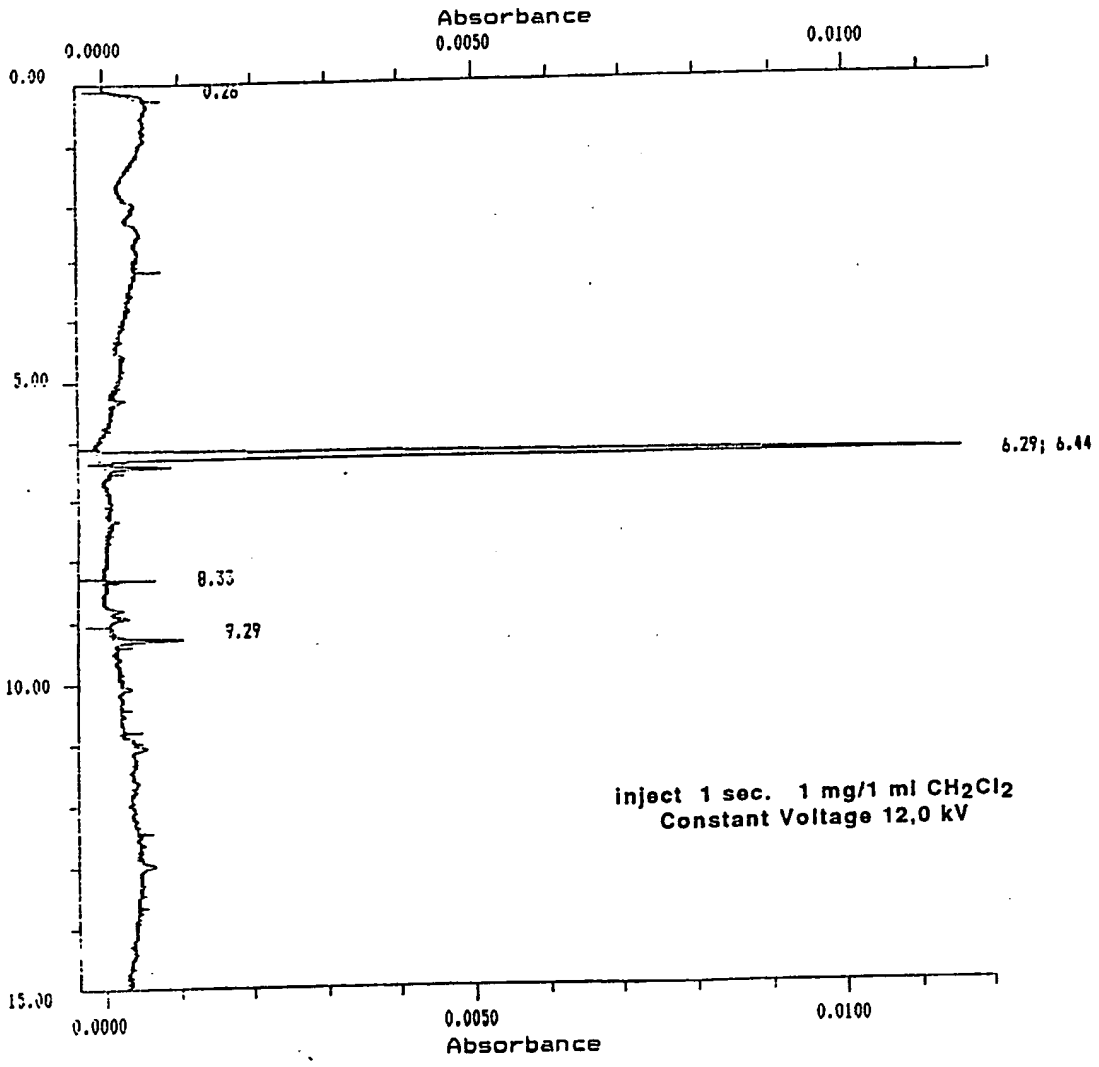
PHARMACOKINETICS I

Analytical detection of ERGOSTERYL-all trans-RETINATE

Active substance, pure, as STANDARD

METHOD: Capillary Zone Electrophoresis

FIGURE 3



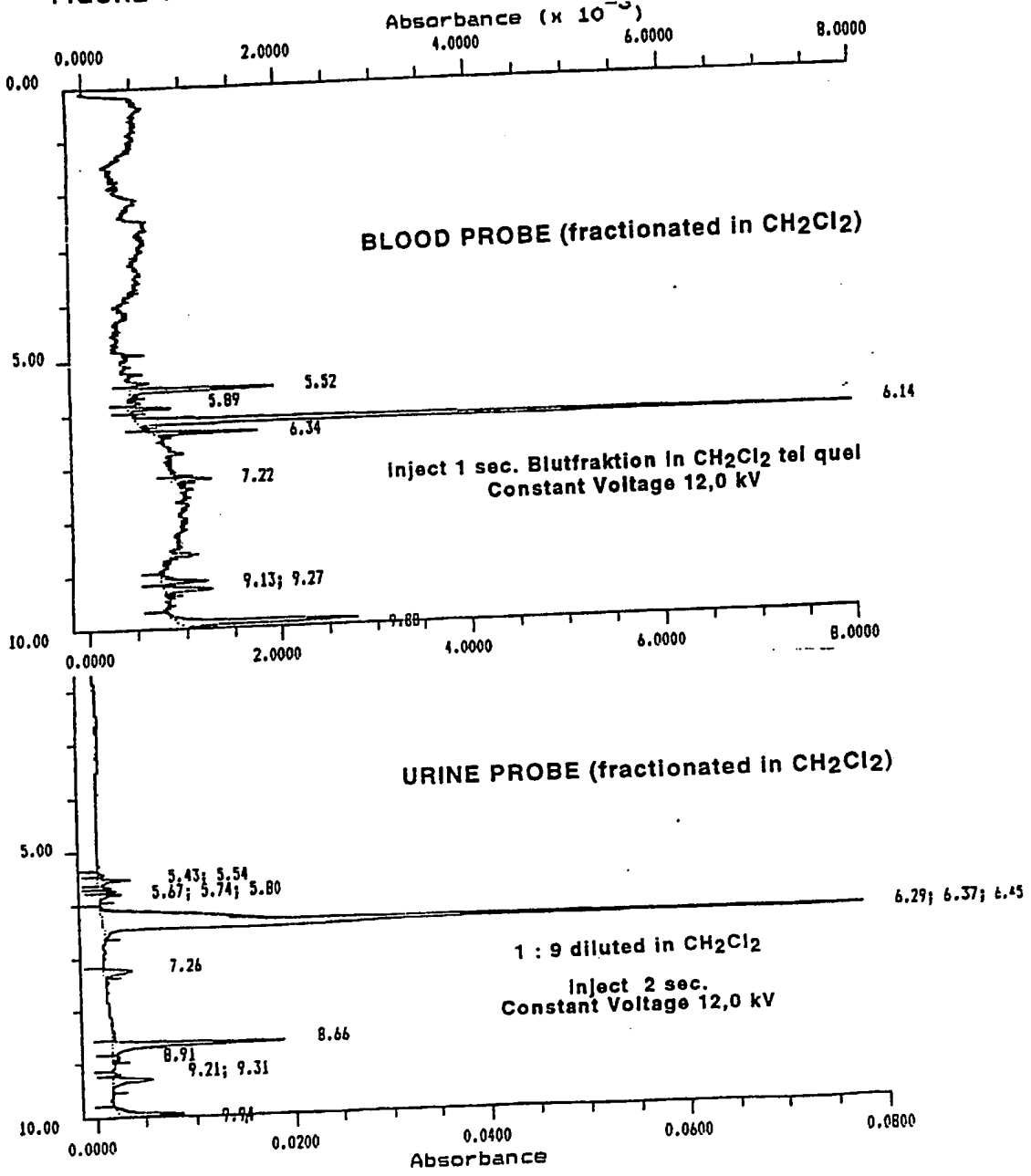
P/ACE SYSTEM 2000 VERSION 1.50 Beckman Instruments Inc.

PHARMACOKINETICS II

Analytical detection of ERGOSTERYL-all trans-RETINATE-CONCENTRATE in the human BLOOD and URINE after oral adminsitration of the CONCENTRATE

METHOD: Capillary Zone Electrophoresis

FIGURE 4



2285805

MARIGEN S.A.

CASE 160

ESTERS with DICARBONIC ACIDS having ANTITUMOUR PROPERTIES

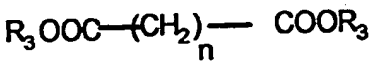
INTRODUCTION

The present invention concerns new esters having antitumor activity, of saturated and unsaturated dicarbonic acids with sterols, vitamin-D and vitamin-E compounds, processes for their production as well as for the preparation of spontaneously dispersible concentrates and pharmaceutical compositions containing these compounds, and their use for the treatment of tumors.

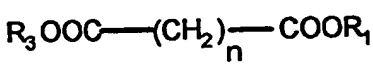
Surprisingly it has been found that *the newly synthetized esters of saturated and unsaturated dicarbonic acids with sterols, vitamin-D and vitamin-E compounds possess outstanding antitumour properties,* particularly if these compounds are being incorporated into spontaneously dispersible concentrates.

DESCRIPTION OF THE INVENTION

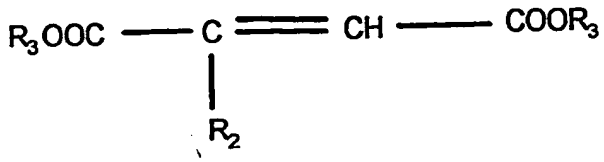
The new esters of saturated and unsaturated dicarbonic acids with sterols, vitamin-D and vitamin-E compounds correspond to the general formulae (I) to (VI) :



(I)



(II)



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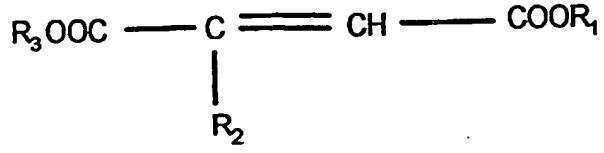

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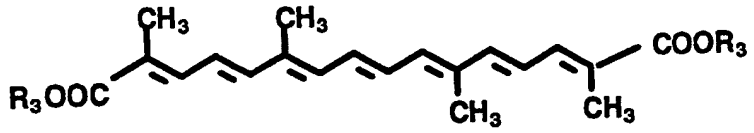
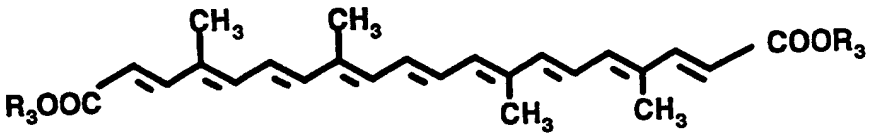
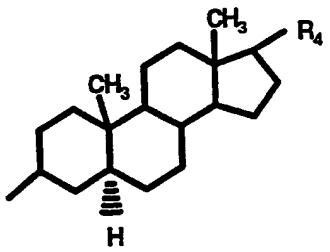


(V)



(VI)

whereby in the formulae (I) to (II) the letter n stands for the numbers 1 to 18, and in the formulae (I) to (VI) R_1 designates a C_1 to C_{20} alkyl or C_2 to C_{20} alkenyl group, R_2 means hydrogen or methyl and R_3 represents one of the radicals of the following formulae:



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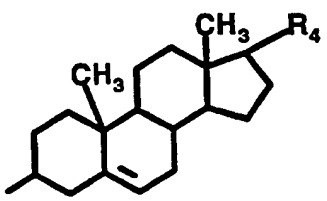

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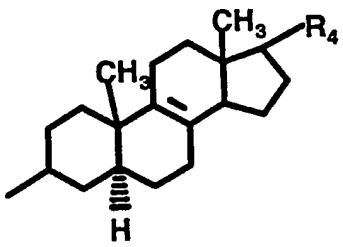

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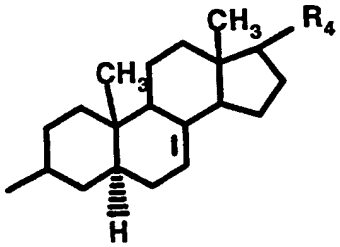
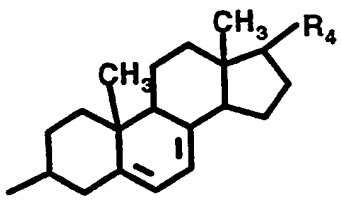


(X)



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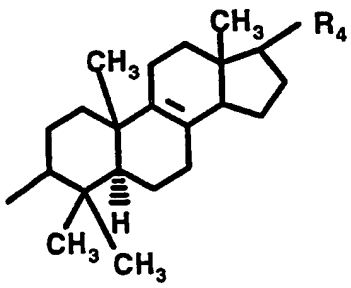

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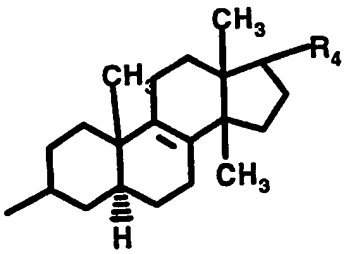

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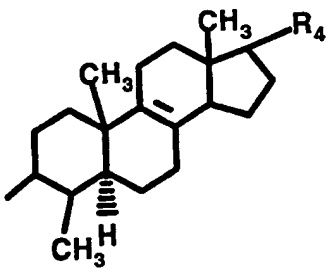
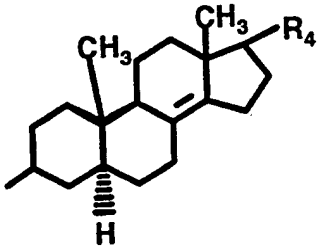
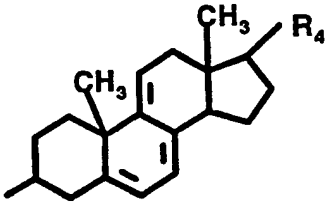


(XIV)

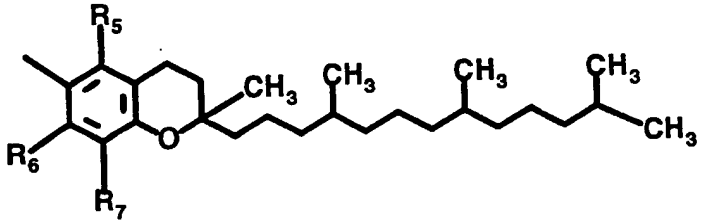


(XV)

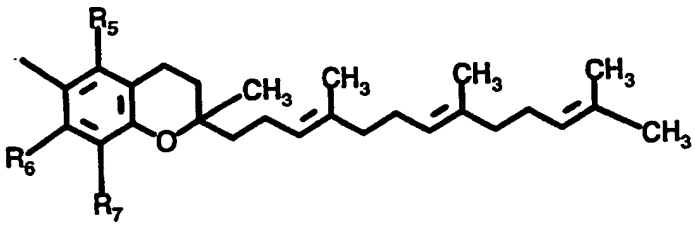


(XVI)

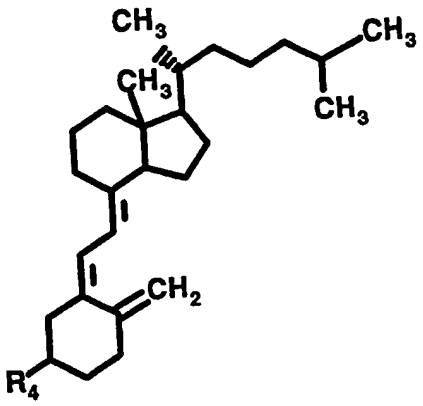
whereby in the formulae (VII) to (XVI) the radical R_4 stands for a C_1 to C_{10} alkyl or a C_2 to C_{10} alkenyl group,



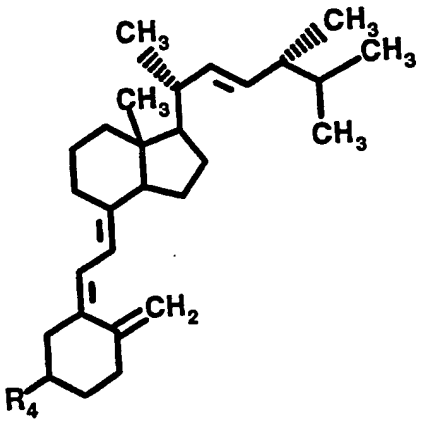
(XVII)



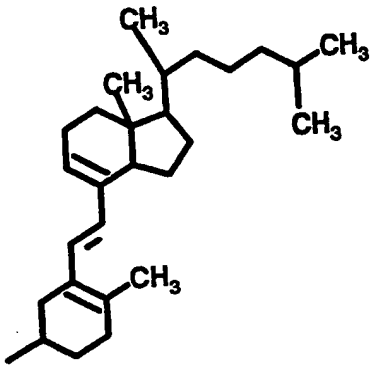
(XVIII)



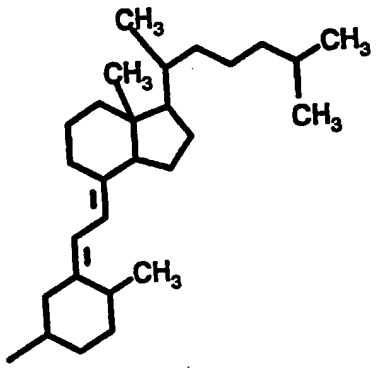
(XIX)



(XX)

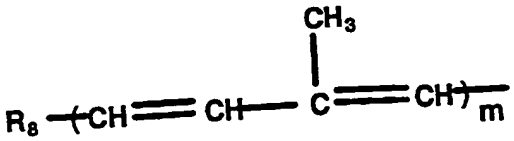


(XXI)

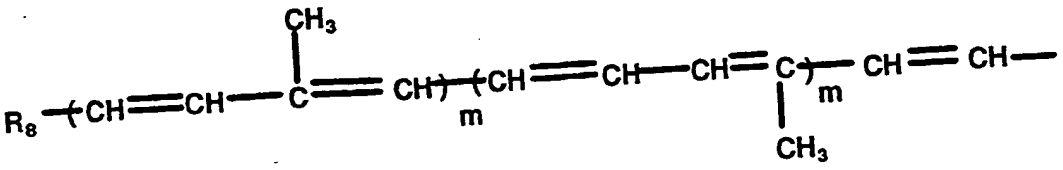


(XXII)

and where in the formulae (XVII) and (XVIII) the radicals R_5, R_6 und R_7 represent hydrogen or methyl,

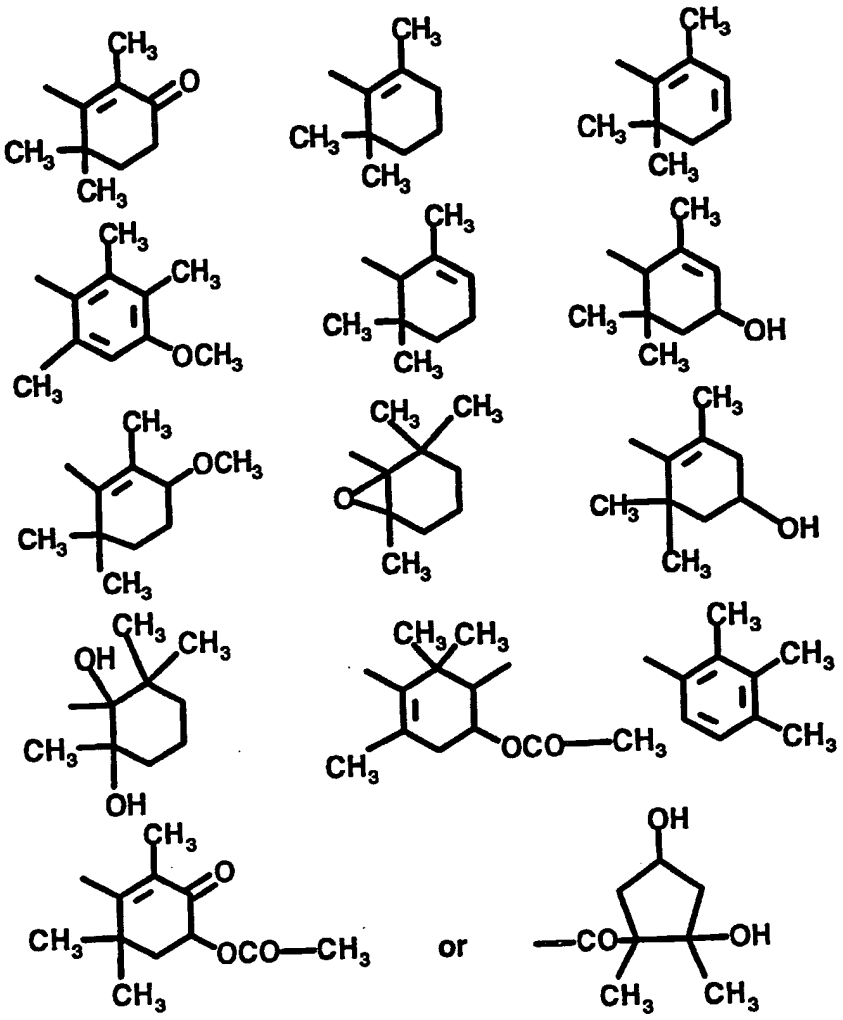


(XXIII)



(XXIV)

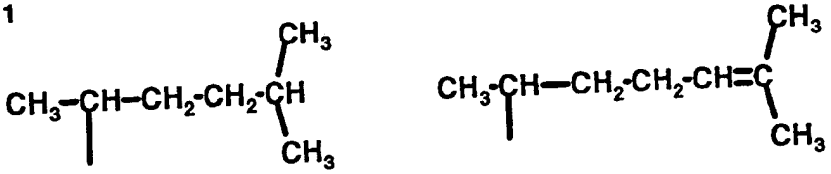
whereby in the formulae (XXIII) and (XXIV) the letter m means the numbers 1, 2, 3, 4 or 5 and R_8 stands for one of the radicals of formulae:



The alkyl and alkenyl groups at R_1 and R_4 can be straight-chained or branched. At R_1 the alkyl groups preferably have 1 to 4 carbon atoms.

At R_4 in the formulae (VII) to (XVI) the alkyl and alkenyl groups normally have 8 to 10 carbon atoms. Examples of such compounds are, inter alia:

1



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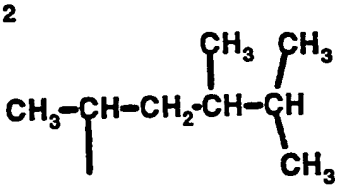

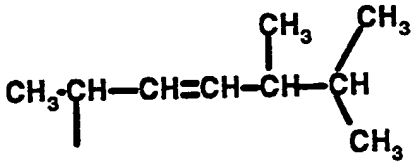

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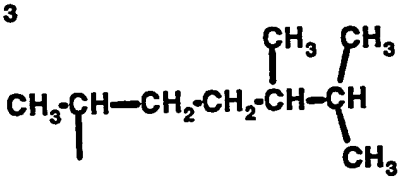

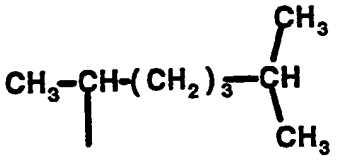

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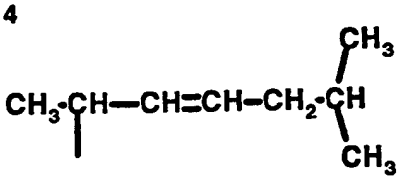

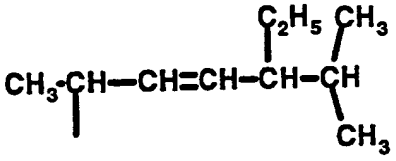

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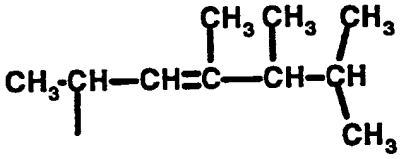

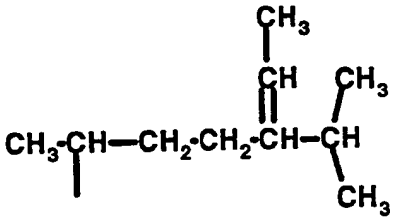

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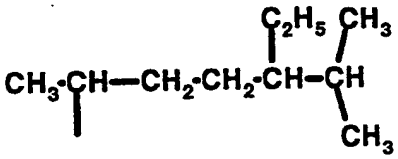

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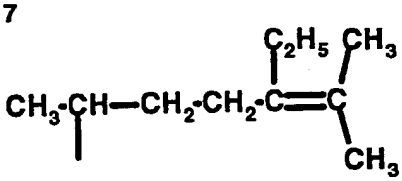
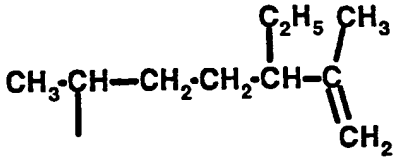




In accordance with the starting products maleic acid and fumaric acid respectively, the compounds of formulae (III) and (IV) have either a cis- or a trans-form.

The class of the vitamin-D compounds comprises the following chemical compounds:

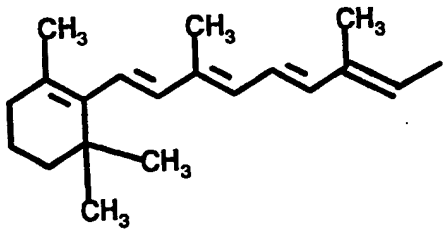
Cholecalciferol: (5Z, 7E)-(3S)-9,10-seco-5,7,10 (19)-cholestatrien-3-ol

25-hydroxycholecalciferol [Calcidiol]:
(5Z, 7E)-(3S)-9,10-seco-5,7,10 (19)-cholestatrien-3,25-diol
1a-dehydroxy-cholecalciferol [Calcitriol] :
(5Z, 7E)-(1S, 3R)-9,10-seco-5,7,10 (19)-cholestatrien-1,3,25-triol
Ergocalciferol [Calciol] :
(5Z, 7E, 22E)-(3S)-9,10-seco-5,7,10 (19),22-ergostatetraen-3-ol
Tachysterol: (6E)-(3S)-9,10-seco-5, (10)-6,8-cholestatrien-3-ol
Dehydrotachysterol: (5E, 7E)-(3S, 10S)-9,10-seco-5,7-cholestatrien-3-ol.

The class of the vitamin-E compounds comprises all tocol- and tocotrienol-compounds, such as, e.g.:

Tocol [2-methyl-2(4,8,12-trimethyltridecyl)chroman-6-ol]
α-Tocopherol [5,7,8-trimethyltocol],
which can take on the following configurations:
12,12,12-α-tocopherol
2-epi-α-tocopherol
2-ambo-α-tocopherol
all-rac-α-tocopherol
4-ambo-8-ambo-α-tocopherol
β-Tocopherol [5,8-dimethyltocol]
γ-Tocopherol [7,8-dimethyltocol]
δ-Tocopherol [8-methyltocol]
Tocotrienol-2-methyl-2-(4,8,12-trimethyldeca-3,7,11-trianyl)chroman-6-ol]
ρ1- oder ρ2-Tocopherol [5,7,8-trimethyltocotrienol] and
ε-Tocopherol [5,8-dimethyltocotrienol or ε-tocotrienol]

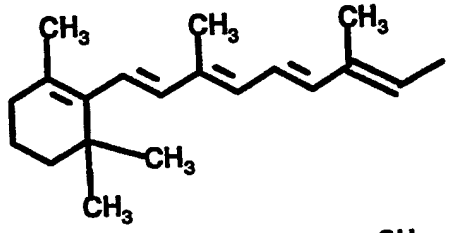
The most important radical according to formula (XXIII) is characterized by formula (XXV) :



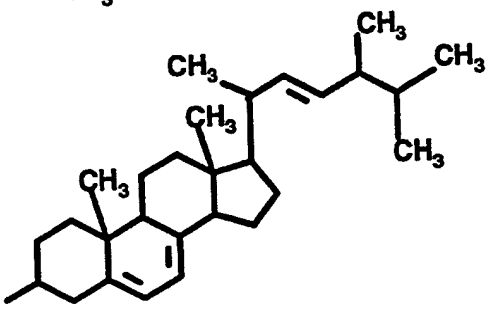
(XXV)

This compound can be present in diverse stereoisomeric forms, such as e.g. all trans, 9-cis or 13-cis.

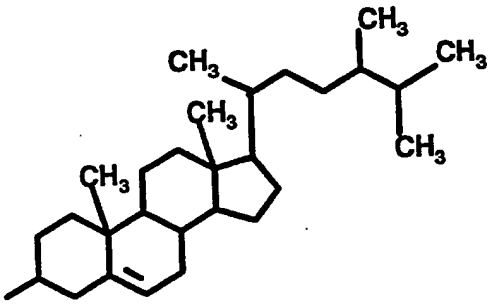
Of particular importance are compounds of the formulae (I) to (VI), in which the letter n represents the numbers 1 to 8, R_1 stands for methyl or ethyl, R_2 signifies hydrogen and R_3 one of the radicals of the formulae (XXV) to (XXX)



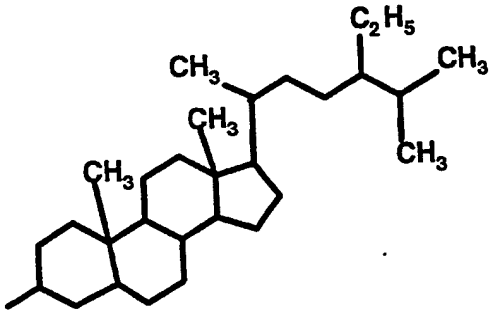
(XXV)



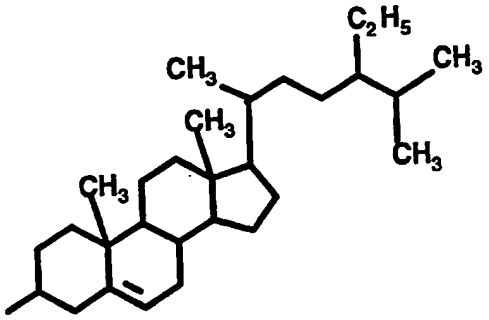
(XXVI)



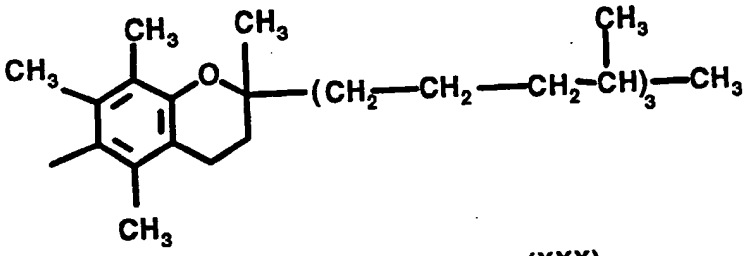
(XXVII)



(XXVIII)



(XXIX)



(XXX)

Examples of inventive new double esters according to formulae (I) to (VI) are, inter alia :

Malonic acid bis-Stigmasterylester
Succinic acid bis-Stigmasterylester
Glutaric acid bis-Stigmasterylester
Adipic acid bis-Stigmasterylester
Pimelic acid bis-Stigmasterylester
Suberic acid bis-Stigmasterylester
Azelaic acid bis-Stigmasterylester
Sebacic acid bis-Stigmasterylester

Azelaic acid bis-β-Sitosterylester
Sebacic acid bis-β-Sitosterylester
Azelaic acid bis-Ergosterylester
Sebacic acid bis-Ergosterylester
Azelaic acid bis-Cholesterylester
Sebacic acid bis-Cholesterylester

Maleic acid bis-Stigmasterylester
Fumaric acid bis-Stigmasterylester
Maleic acid bis-β-Sitosterylester
Fumaric acid bis-β-Sitosterylester

Maleic acid bis-Ergosterylester
Fumaric acid bis-Ergosterylester
Maleic acid bis-Cholesterylester
Fumaric acid bis-Cholesterylester

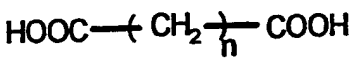
Azelaic acid Calciferyl-diester
Sebacic acid Calciferyl-diester
Azelaic acid Cholecalciferyl-diester
Sebacic acid Cholecalciferyl-diester
Azelaic acid DL-α-Tocopheryl-diester
Sebacic acid DL-α-Tocopheryl-diester

Glutaric acid ethyl-Calciferylester
Glutaric acid Calciferyl-methylester
Fumaric acid ethyl-Calciferylester
Fumaric acid Calciferyl-methylester
Azelaic acid ethyl-Calciferylester
Azelaic acid Calciferyl-methylester
Azelaic acid ethyl-Cholecalciferylester
Azelaic acid Cholecalciferyl-methylester
Sebacic acid ethyl-Calciferylester
Sebacic acid Calciferyl-methylester
Sebacic acid ethyl-Cholecalciferylester
Sebacic acid Cholecalciferyl-methylester

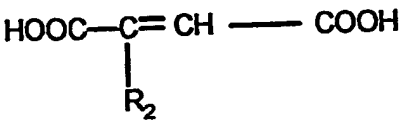
PROCESSING

The compounds according to formulae (I), (III), (V) and (VI) can be manufactured according to the following procedures, which are known per se:

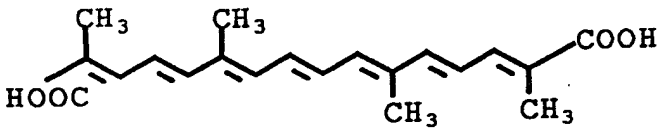
a) Reaction of a compound of formulae (XXXI) to (XXXIV)



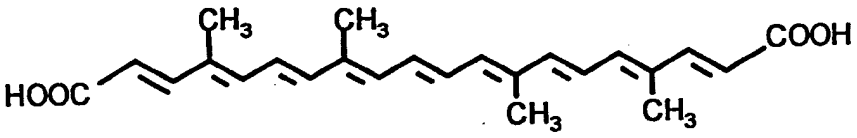
(XXXI)



(XXXII)



(XXXIII)



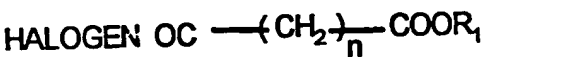
(XXXIV)

in which the letter n represents the numbers 1 to 18 and R_2 means hydrogen or methyl, with 1,1'-carbonyldiimidazole (CDI) at 25 to 70 °C under addition of a catalytic amount of an alcoholate in an indifferent solvent, such as tetrahydrofuran, benzene, toluene or chloroform, followed by alcoholysis of the imidazolides formed with a sterol, a vitamin-D or vitamin-E compound.

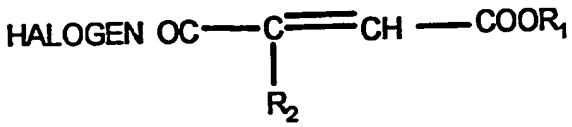
b) Formation of the dichloride or the dibromide of a compound of the formulae (XXXI) to (XXXIV) with a chlorination or bromination agent such as thionylchloride, oxalylchloride or oxalylbromide, followed by the reaction of the dichloride or dibromide formed with a sterol, a vitamin-D or a vitamin-E compound at a temperature of between 40 and 80 °C in an indifferent solvent such as toluene or tetrahydrofuran, and in the presence of a catalyst such as dimethylformamide or p-dimethylaminopyridine.

c) Procedure for the preparation of the compounds according to formulae (II) and (IV).

Reaction of a compound of formulae (XXXV) and (XXXVI) :



(XXXV)



(XXXVI)

in which the letter n determines the numbers 1 to 18, R_1 stands for alkyl or alkenyl and R_2 means hydrogen or methyl, with a sterol, a vitamin-D or a vitamin-E compound at a temperature of between 40 and 80 °C in an indifferent solvent such as toluene or tetrahydrofuran, and in the presence of a catalyst such as dimethylformamide or pyridine.
The compounds of formulae (XXXV) and (XXXVI) are prepared in known manner by first producing the dihalogenide of the formulae (XXX) and (XXXI) respectively according to b) and then reacting the formed dihalogenides with a half-stechiometric amount of an alkyl or alkenyl alcohol in the presence of a catalyst such as e.g. dimethylformamide or pyridine, in order to obtain the halfester of the inventive compounds, followed by purification by means of distillation or chromatography.

The *novel dicarbonic acid esters according to formulae (I) to (VI)* surprisingly possess excellent *antitumour activity*. They are particularly effective against tumors of the skin and of epithelial tissues. most notably in case when these compounds have been incorporated into *spontaneously dispersible concentrates*. For this reason, *spontaneously dispersible con-centrates* made up with the *new dicarbonic acid esters* according to the formulae (I) to (VI) are also a subject matter of the present invention.
When treated with water, such concentrates render microemulsions having excellent phase stability as well as much improved membrane permeability and spreading properties. Control measurements, executed at the Swiss Federal Institute of Technology, Zurich (Institute for Polymers, Biopolymers, Prof. Dr. Pier Luigi LUISI and Prof. Dr. Peter SCHURTENBERGER) showed that the generated micelles have a hydrodynamic radius of 1,2 to 2,4 nm.

All experimental observations gained with stable microemulsions of this kind can be uniformly interpreted by means of the *assumption* that the system produces in the aqueous phase *organized aggregates*, which are called

All experimental observations gained with stable microemulsions of this kind can be uniformly interpreted by means of the *assumption* that the system produces in the aqueous phase *organized aggregates*, which are called MICELLES. These micelles possess a more or less globular shape, having a hydrodynamic radius of less than 10 nm. They are thermodynamically stable. The tensides and cotensides are capable at the phase-boundary of the microemulsion of hindering SELF-DIFFUSION. This means that no mixing takes place between the outer aqueous phase of the microemulsion and the inner oily phase, which contains the dicarbonic acid ester compounds solubilized in the coemulgator and/or biotenside solvent.

The micelles, which contain in their inner phase the solubilized antitumor substances, are coated by a tenside layer or bilayer and are thus enabled first to penetrate the human skin and then to penetrate through the plasma membrane of the tumour cell. This diffusion process takes place on account of thermal molecular movements (Brownian movement) exclusively.

The volume and the speed of substance transport across the cell membranes are dependent upon the differential in concentration existing between the extracellular outside and the inside of the individual tumour cell. The diffusion flow continues along the concentration gradient until it is consumed and an equal concentration of active substance [or of a therapeutic system] is reached in both compartments, the extracellular zone and the internal zone. Such diffusion processes occur independently of any energy input from outside into the interacting compartments. They can show slow-release-effects. In biological systems they are not related to metabolic energy.

The speed of diffusion is governed by Fick's law of diffusion

$$\frac{dm}{dt}\ \frac{1}{q} = -D\ \frac{dc}{dx} \qquad \text{EQUATION (A)}$$

where dm signifies the amount in Mol of active substance molecules which penetrate a cell surface q (in cm^2) per time-unit dt (in seconds). D is the coefficient of diffusion and dc the concentration differential over the distance dx.

According to NERNST the diffusion coefficient is dependent on the absolute temperature and friction resistance

$$D = \frac{R}{N}\,\frac{T}{f} = \frac{kT}{f} \qquad \text{EQUATION (B)}$$

Friction resistance is, according to STOKE's law,

$$f = 6\,\pi\,\eta\,r \qquad \text{EQUATION (C)}$$

a function of the viscosity of the diffusing solution and of the *radius of the diffusing particles.* By substituting f with $f = 6\,\pi\,\eta\,r$ in the Nernst equation, one obtains the SUTHERLAND-EINSTEIN equation for the DIFFUSION COEFFICIENT

$$D = \frac{RT}{N}\,\frac{1}{6\,\pi\,\eta\,r} = \frac{kT}{6\,\pi\,\eta\,r} \qquad \text{EQUATION (D)}$$

where k stands for the Boltzmann constant.

If for a particular diffusion process one assumes a regular reduction of concentration in the membrane of the tumor cell, then the expression $\frac{dc}{dx}$ in the diffusion law can be restated as $\frac{\Delta c}{x}$ (= concentration difference Δc over a membrane of thickness x). x is a constant value for a specific membrane. For this reason, it can be combined with the diffusion coefficient to express a new constant, the *permeability coefficient P :*

$$P = \frac{D}{x} \qquad \text{EQUATION (E)}$$

The expression $\frac{dm}{dt}\,\frac{1}{q}$ in the diffusion equation is called FLUX J. It has the dimension Mol per second per cm^2. The negative sign on the right side of the equation indicates that the transport of the molecules of the active substance or the systems preparation containing active substances flows in the direction of the decreasing concentration.

Therefore, we have

$$J = -F\Delta c = -\frac{RT}{Nx}\,\frac{1}{6\,\pi\,\eta\,r}\,\Delta c = \frac{kT}{x}\,\frac{1}{6\,\pi\,\eta\,r}\,-\Delta c$$

EQUATION (F)

It can be deduced from this equation that the velocity of the diffusion process across the cell membrane is governed by:

1) the concentration difference Δc in the two compartments
2) the radius of the particles of the diffusing active substance or system's preparation
3) the viscosity of the diffusing aqueous solution (emulsion)
4) the temperature

The *spontaneously dispersible concentrates* prepared in accordance with the invention contain
0.001 to 25 % by weight of individual esters with dicarbonic acids according to formulae (I) to (VI), and combinations of such compounds respectively
0.001 to 40 % by weight of a solvent or solvent mixture which is pharmaceutically acceptable and acts as a hydrotropic or coemulsifier
0.001 to 90 % by weight of a pharmaceutically acceptable surfactant or surfactant mixture, and optionally
up to 10 % by weight of a vitamin or provitamin
up to 10 % by weight of a free fatty acid, and if appropriate, customary excipients and/or diluents.

The surfactants or surfactant mixtures to be employed according to the invention can be anionic, cationic, amphoteric or non-ionic. Ideally, they are non-ionic and have an HLB-value (i.e. a hydrophilic-lipophilic balance) of between 2 and 18; preferably, it is between 2 and 6 on the one hand and 10 and 15 on the other hand. HLB values describe the hydrophilic and lipophilic properties of an emulsifier. In this context see "Hydrophile-Lipophile Balance: History and recent Developments" by Paul Becher in Journal of Dispersion Science and Technology, 5 (1), 81-96 (1984).

Suitable anionic surfactants can be both socalled water-soluble soaps and water-soluble synthetic compounds.
Suitable soaps are the alkali metal salts, alkaline earth metal salts or optionally substituted ammonium salts of higher fatty acids (C_{12} to C_{22}), for example the natural Na or K salts of oleic or stearic acids, or of natural mixtures of fatty acids which can be obtained, inter alia, from coconut oil or tallow oil. Other surfactants which may be mentioned are fatty acid methyltaurine salts, and modified and non-modified phospholipids.

However, more frequently used surfactants are so-called synthetic surfactants, in particular fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives or alkylaryl-sulfonates.
The fatty sulfonates and fatty sulfates are usually present in the form of alkali metal salts, alkaline earth metal salts or optionally substituted ammonium salts and generally have an alkyl radical containing 8 to 22 C atoms, alkyl also encompassing the alkyl moiety of acyl radicals. Examples are the Na or Ca salt of ligninsulfonic acid, of dodecylsulfuric ester and

sulfonic acids of fatty alcohol/ethylene oxide adducts. The sulfonated benzimidazole derivatives preferably contain two sulfonyl groups and one fatty acid radical containing about 8 to 22 C atoms. Alkylarylsulfonates are, for example, the Na, Ca or triethanolamine salts of dodecylbenzenesulfonic acid, of dibutylnaphthalene-sulfonic acid or of a naphthalenesulfonic acid/formaldehyde condensation product.

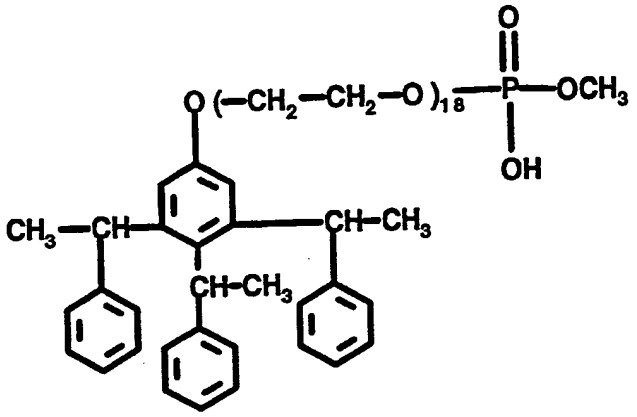
The non-ionic surfactants are mainly chosen from amongst the polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, saturated or unsaturated fatty acids and alkylphenols which can contain 3 to 30 glycol ether groups and 8 to 20 C atoms in the (aliphatic) hydrocarbon radical and 6 to 18 C atoms in the alkyl radical. Other suitable non-ionic surfactants are the water-soluble polyethyleneoxy-adducts onto polypropylene glycol and alkyl polypropylene glycol with 1 to 10 C atoms in the alkyl chain, which adducts contain 20 to 250 ethylene glycol ether groups and 10 to 100 propylene ether groups. The compounds which have been mentioned customarily contain 1 to 5 ethylene units per propylene glycol unit.

The following may be mentioned as examples of non-ionic surfactants: nonylphenol polyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxy-polyethoxy-ethanol, polyethylene-glycol and octylphenoxy-polyethoxyethanol. Moreover, fatty acid esters of polyoxyethylene-sorbitan, such as polyoxyethylene sorbitan trioleate, are also suitable.

The cationic surfactants are mainly quaternary ammonium salts which contain at least one alkyl radical having 8 to 22 C atoms as the N-substituent and which have lower, optionally halogenated alkyl radicals, benzyl radicals or lower hydroxyalkyl radicals as further substituents. The salts are mainly present in the form of halides, methylsulfates or ethylsulfates, for example stearyltrimethylammonium chloride or benzyldi-(2-chloroethyl)-ethyl-ammonium bromide.

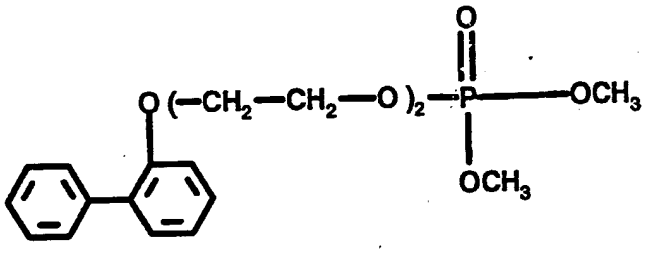
When preparing the inventive spontaneously dispersible concentrates, special preference is given on the one hand to phosphoric acid ester tensides, such as:

Tristyrylphenolpolyoxyethylene-18-mono/dimethyl-phosphoric-acid-ester (Soprophor® FL, Rhône-Poulenc);



Soprophor FL (Rhône-Poulenc)

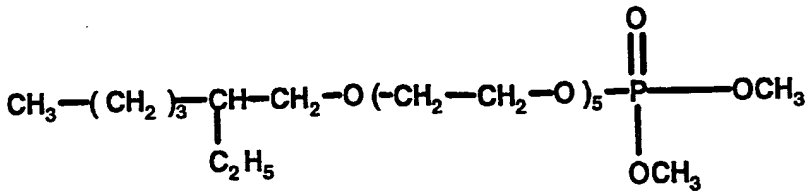
Nonylphenol-10-polyoxyethylene-mono/dimethylphosphoric-acid-ester (Diphasol® 3873, CIBA-GEIGY); or the identical Sermul® EA 188 (SERVO)



(Tensid 508, CIBA-GEIGY);

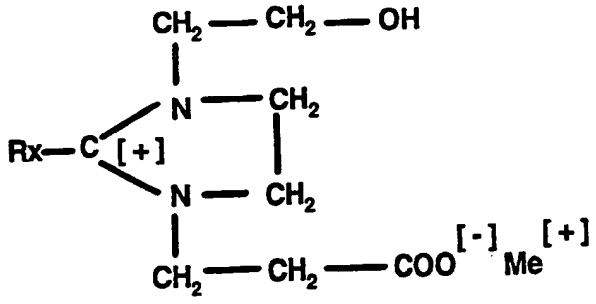
Tinovetin® JU (CIBA-GEIGY), a hydroxybiphenyl-10-ethoxy-phosphoric acid ester

Butyl-mono-4-ethoxy-phosphoric acid ester (Zerostat® AT, CIBA-GEIGY), and



(Zerostat ® AN , CIBA-GEIGY), respectively

and on the other hand to betain compounds, i.e. amphoteric, salt- and waterfree imidazole derivatives, having an isoelectric/isoionic point near 7, such as e.g.



in which Me[+] stands for hydrogen, alkali and/or earth alkali atoms, and R_x for a C_1 to C_{32} alkyl or C_2 to C_{32} alkenyl group.
Furthermore, so-called "multi-functional glucose derivatives", such as, e.g., Glucate® SS (Methyl-glucose-sesquistearate) and Glucamate® SSE-20 (PEG-20-methyl-glucose-sesquistearate) of Amerchol, Edison, N.J., are also being used.

The following compounds may be employed as the pharmaceutically acceptable solvent which acts as the hydrotropic, or coemulsifier, for example: esters of an aliphatic alcohol (C_3 to C_{18}) with an aliphatic carboxylic acid (C_{10} to C_{22}), such as isopropyl laurate, hexyl laurate, decyl laurate, isopropyl myristate and lauryl myristate; hydrocarbons having a straight carbon chain (C_{12} to C_{32}) which is substituted by 6 to 16 methyl groups and which can have up to 6 double bonds, examples which may be mentioned being terpenes, such as polymethylbutanes and polymethyl-butenes.
Monoesters of ethylene glycol or propylene glycol with an aliphatic carboxylic acid (C_6 to C_{22}), such as propylene glycol monolaurate and propylene glycol monomyristate.
Esters of an aliphatic alcohol (C_{12} to C_{22}) with lactic acid, such as, for example, myristyl lactate or, preferably, lauryl lactate. Monoesters or diesters of glycerol with an aliphatic carboxylic acid (C_6 to C_{22}), such as, for example, glyceryl caprylate or Miglyol® 812 neutral oil (Oleum neutrale).
Esters of a poly(2-7)ethylene glycol glycerolether having at least one free hydroxyl group with an aliphatic carboxylic acid (C_6 to C_{22}), such as, for example, aliphatic alcohols (C_{12} to C_{22}), thus, inter alia, dodecanol, tetra-dodecanol, oleyl alcohol, 2-hexyldecanol and 2-octyl-decanol.
Esters containing at least one free hydroxyl group, of poly-(2-10)glycol with an aliphatic carboxylic acid (C_6 to C_{22}), monoethers of a polyethylene

with an aliphatic alcohol (C_{12} to C_{18}), such as, for example, poly-oxyethylene-(C10) octylether.
Heterocyclic compounds such as 1-methyl-2-pyrrolidone.
Biotenside esters according to the general formula:

$$R^2—COO—R^3$$

in which R^2 stands for citronellyl, geranyl, farnesyl, phytyl or isophytyl and R^3 means a C_1 to C_{32} alkyl amd a C_2 to C_{32} alkenylgroup respectively.

Before their application in the spontaneously dispersible concentrates all technical tensides have been cleaned by filtration or by chromatography over aluminum-oxide with an inert solvent as eluent, such as tetra-hydrofurane, ethyl alcohol or dichloromethane.

Suitable additives for the spontaneously dispersible concentrates according to the invention are vitamins and provitamins [such as, for example, vitamin A (retinoic acids), retinol, carotenes, tocopherols], and also free fatty acids, such as: valeric acid, isovaleric acid, sorbic acid, isocaproic acid, pelargonic acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, hexacosanoic acid, octacosanoic acid, pentadecanoic acid, decenylic acid, undecylenic acid, dodecenylic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, erucic acid, etc.

The daily dose required for pharmaceutical administration is 0.001 to 25 mg/kg of body weight, if possible split into 2-3 individual doses. For this purpose, the new vitamin-sterol esters sterol phosphatides, or the spontaneously dispersible concentrates with these compounds, can be incorporated into the conventional pharmaceutical preparations and dosage forms, such as coated tablets, tablets, capsules, powders, granules, pellets, solutions, ampuls, emulsions, creams or suppositories together with the customary excipients and/or diluents and stabilizers.
The active substances or mixtures of active substances which form the subject-matter of the invention, and the spontaneously dispersible concentrates which contain these active substances or mixtures of active substances, can be administered to humans orally, by injection (intravenously, subcutaneously or intramuscularly) or in other ways. If they are presented as solid dosage forms for oral administration, this can be in the

form of tablets, granules, pellets, powders or capsules, etc. The preparations can contain additives, for example a pharma-ceutical excipient, such as a saccharide or cellulose base, a binder, such as starch paste or methylcellulose, a filler, or a disintegrant, etc., with additives being employed which are customarily used in the preparation of medicinal or pharmaceutical formulations. When the active substances or mixtures of active substances according to the invention are administered orally in the form of liquid dosage forms, they can be present in any form selected from amongst aqueous preparations for internal use, from suspensions, emulsions and syrups, etc., and they can also be present in the form of dried preparations which are dissolved or emulsified prior to use.
When the active substances or mixtures of active substances according to the invention are processed in the form of aqueous solutions, suspensions or oily or aqueous emulsions, preferably microemulsions, from the spontaneously dispersible concentrates according to the invention, they can also be injected. However, it is customary to prepare the injection solutions shortly before administration, by dissolving or suspending the extracts or concentrates in aqueous, liquid media, such as sterile water or physiological sodium chloride solution or glucose solution.
If required, conventionally used solvents, stabilizers, preservatives and additives for the preparation of isotonic solutions can be added to a preparation for injection. The preparations for injection obtained in this manner are administered intravenously, intramuscularly, subcutaneously or in any other suitable way.

The present invention also relates to pharmaceutical preparations which contain the active substances, or mixtures of active substances, or the spontaneously dispersible concentrates, which have been above described, for controlling the growth of tumour cells. The pharmaceutical preparations according to the invention are those which can be used for enteral (such as oral or rectal) or for parenteral or topical administration to warm-blooded animals, which preparations contain the spontaneously dispersible concentrate on its own or together with a pharmaceutically acceptable excipient.
The dosage of the concentrates according to the invention depends on the warm-blooded species, on the age and on the individual condition, and on the mode of administration. For example, doses in the range of about 0.1 - 50 mg/kg of body weight are administered subcutaneously, and doses in the range of 0.05 - 5 mg/kg of body weight are administered intraperitoneally to

warm-blooded animals having a low body weight, such as, for example, mice, rats and hamsters, to achieve an effect of tumour cell destruction.

The oral and rectal forms of the novel pharmaceutical preparations contain between 1 and 95 %, preferably between 10 and 95 %, and in particular between 20 and 95 %, of the spontaneously dispersible concentrate according to the invention. For example, they can be present in unit-type dosage forms, i.e., as coated tablets, micropellets, tablets, suppositories or ampuls and, in particular, as capsules.

Suitable pharmaceutically acceptable excipients for the oral forms are mainly fillers, such as sugars (for example lactose, sucrose, mannitol or sorbitol), cellulose preparations and/or calcium phosphates (for example tricalcium phosphate or calcium hydrogen phosphate), furthermore bin-ders, such as starch paste, with the use of, inter alia, corn starch, wheat starch, rice starch or potato starch, gelatine, tragacanth, methylcellulose, hydroxymethylcellulose, sodium carboxy-methylcellulose and/or polyvinyl-pyrrolidone and/or disintegrants (if desired), such as the above mentioned starches, furthermore carboxymethyl starch, crosslinked polyvinylpyrroli-done, agar, alginic acid or a salt thereof, for example sodium alginate. Examples of suitable flow-control agents are the polyethylene glycols Nos. 200 - 600 and above.

The gelatine capsules, which are still the preferred dosage form for hu-mans, are provided with suitable coatings, concentrated sugar solutions [which can optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide], lacquer solutions (aqueous or those which have been prepared using organic solvents), or enteric coatings of solutions of suitable cellulose preparations, such as microcrystalline cellulose (Avicel®), acetyl-cellulose phthalate, hydroxymethylcellulose-phthalate, Metolose® , AQOAT® or a copolymer, such as Eudragit® L 30 D, being used, inter alia.

Pharmaceutical dosage forms for oral use which are particularly suitable according to the invention are two-piece gelatine capsules with a plasticizer, such as glycerol or sorbitol. The soft-gelatine or hard-gelatine capsules and the capsules made of AQOAT® (hydroxypropyl methylcel-lulose) respectively can contain the spontaneously dispersible concentrate

according to the invention as a mixture with fillers, such as lactose, binders, such as starch, and/or glidants, such as talc or magnesium stearate, and, if appropriate, together with stabilizers and antioxidants, such as, for example, α-, β- or γ-tocopherol. It may be expedient to employ suitable liquids, such as liquid polyethylene glycols Nos. 200 to 600 as diluents, to which stabilizers and antioxidants can also be added.

For parenteral administration, distilled water is added to the concentrates according to the invention. To the aqueous microemulsion for injection which then forms, there can be added viscosity-increasing substances, for example Na-carboxymethyl-cellulose, sorbitol, mannitol and/or dextran, and if appropriate also stabilizers and antioxidants.
The pharmaceutical preparations for parenteral administration preferably contain between 0.1 and 60 %, especially between 1 and 40 %, of the spontaneously dispersible concentrate according to the invention.
Suitable preparations for topical use, which are particularly suitable for the prophylaxis and the treatment of cancers of the skin, are, for example, creams, ointments, pastes, foams, tinctures and solutions, which contain between 0.001 and 70 % of the concentrate according to the invention.
Oily bases which are used for creams and oil-in-water emulsions which contain more than 50 % water, are mainly fatty alcohols, for example lauryl alcohol, cetyl alcohol or stearyl alcohol, waxes of liquid to solid consistency, for example isopropyl myristate, wool wax or beeswax and/or hydrocarbons, such as, for example, petroleum jelly (petrolatum) or paraffin oil. Substances which are mainly suitable for emulsifying these oily bases are surface-active, pharmaceutically acceptable substances having predominantly hydrophilic properties, such as, for example, non-ionic emulsifiers, in particular fatty acid esters of polyalcohols or ethylene oxide adducts (such as polyglycerol fatty acid esters or polyethylene sorbitan fatty acid esters) having an HLB value of less than 8. Additives which are added to the water phase are, inter alia, agents which prevent desiccation of the creams, for example polyalcohols, such as glycerol, sorbitol, propylene glycol and/or polyethylene glycols Nos. 200 to 600, and furthermore preservatives, odor-imparting substances, etc.
Ointments are water-in-oil emulsions which contain up to 70 %, but preferably between 20 and 50 %, water or aqueous phases.
Substances which are suitable as the lipid phase are mainly hydrocarbons, for example petroleum jelly, paraffin oil and/or solid paraffins, which

contain hydroxy compounds suitable for improving the water-binding capacity, for example fatty alcohols or esters, such as cetyl alcohol or wool wax alcohols.

In some cases, emulsifiers having an HLB-value of 8 to 16, such as, for example, sorbitan fatty acid esters (such as sorbitan isostearol) are also added. Additives which are added to the water phase are, inter alia, humectants, such as polyalcohols (glycerol, propylene glycol, sorbitol and/or polyethylene glycols No. 200, 400, 600); and furthermore preservatives, odor-imparting substances, etc.

Fatty ointments are anhydrous and chiefly contain hydrocarbons as the base, for example paraffin, petroleum jelly and/or liquid paraffins; moreover natural or partially-synthetic fats, such as, for example, coconut fatty acid triglyceride, furthermore: fatty acid partial esters of glycerol, such as, for example, the fatty alcohols, emulsifiers and/or additives which increase the water-absorption capacity, all of which have been mentioned in connection with the ointments.

Pastes are creams and ointments containing powder constituents which absorb secretions, such as, for example, metal oxides (such as titanium oxide or zinc oxide), and furthermore talc and/or aluminum silicates whose task it is to bind any moisture or discharge which may be present.

Foams are administered from pressurized containers and are oil-in-water emulsions of the spontaneously dispersible concentrates according to the invention which are present in aerosol form, with halogenated hydrocarbons (such as, for example, lower chloro-fluoroalkanes; such as dichlorodifluoromethane and dichlorotetra-fluorethane) being added as propellants. Other substances which may be added are the customary additives, such as preservatives, etc.

The present invention also relates to the *use of the active substances, mixtures of active substances and spontaneuosly emulsifiable concentrates* according to the invention for inhibiting the growth of tumour cells or as prophylactic agents against oncoses in humans and animals, administration preferably being carried out in the dosage forms which correspond to the pharmaceutical preparations described above.

Processing examples for inventive esters with dicarbonic acids according to formulae (I) to (VI) .

1. Process for the preparation of azelaic acid bis ergosterylester

a) To 9,5 g azelaic acid in 100 ml toluene one adds dropwise at 10 °C 15 g of oxalyl chloride (excess) in 50 ml toluene. Stand the solution at 20 °C during 12 hours. Evacuate subsequently the solvent as well as the excess of oxalyl chloride. One obtains 10,5 g azelaic acid dichloride, with a boiling-point of 243 - 248 °C.

b) To 800 mg ergosterol in 50 ml toluene add dropwise 250 mg azelaic acid dichloride, together with 200 mg dimethylformamide. After heating for 2 hours to 50 °C, the solvent is distilled off. The residue is being re-cristallised in acetonitrile/chloroform 2:1.
The azelaic acid bis ergosterylester is obtained, as white crystals, with a melting point of 183,7 °C.

In a corresponding way, also the following compounds can be prepared:

Azelaic acid bis Cholersterylester		mp	138 - 139 °C
	IR	2942 cm-1	ν(CH)
		2868 "	ν (CH)
		1723 "	ν (C=O) Ester
		1467 "	δ (CH)
		1376 "	δ (CH_3)
		1188 "	ν (C-O) Ester
		1010 "	ν (C-O)
Sebacic acid bis Ergosterylester		RI	1.52514
Sebacic acid bis Cholesterylester		mp	172,8 - 173,4 °C
Sebacic acid bis Stigmasterylester		"	158,5 - 160,5 °C
Glutaric acid bis Stigmasterylester		"	168,5 °C
Glutaric acid bis β–Sitosterolester		"	179,9 °C
Glutaric acid bis Cholesterylester		"	196,8 °C
Glutaric acid bis Ergosterylester		"	216,7 °C
Adipic acid bis Cholesterylester		"	194,8 °C
Adipic acid bis Ergosterylester		"	222,5 - 223,5 °C
Adipic acid bis Stigmasterylester		"	216,2 °C
Fumaric acid bis Stigmasterylester		"	277,5 - 278,5 °C
Fumaric acid bis Cholesterylester		"	240,0 - 240,5 °C
Fumaric acid bis Ergosterylester		"	181,4 - 182,8 °C

2. Preparation of Azelaic acid Ergocalciferyldiester.

To 800 mg Ergocalciferol (Calciol) in 40 ml toluoene one adds dropwise at room temperature 250 mg azelaic acid dichloride and 300 mg pyridine in 25 ml toluene. After heating to 50 °C for 1 hour, the solvent is distilled off. The residue is chromatographed on a silicagel column with cyclohexane/ethyl acetate (90:10) as eluent.

The azelaic acid ergocalciferyldiester is obtained, with a refractory index (RI) $n\frac{20}{D}$ of 1,51860.

Infrared spectrum :

IR	2932	cm^{-1}	ν (CH)
	2870	"	ν (CH)
	1721	"	ν (C=O) Ester
	1630	"	ν (C=C)
	1466	"	δ (CH)
	1370	"	δ (CH_3)
	1187	"	ν (C-O)
	958	"	δ (CH) trans C=C of (D_3)

In a similar way, also the following compounds can be prepared:

Azelaic acid retinyl-diester (chromatographed with n-hexane/ethyl acetate 80:20)	RI	1.56858
Azelaic acid Cholecalciferyl-diester	RI	1.54920
Succinic acid Cholecalciferyl-diester	RI	1.53252
Fumaric acid Ergocalciferyl-diester	RI	1.53774
Glutaric acid Ergocalciferyl-diester	RI	1,54224
Azelaic acid Ergocalciferyl-diester	RI	1.52340
Sebacic acid Ergocalciferyl-diester	RI	1.53528

The characteristic ester bindings can be detected in the infrared spectrum at a wave length of ca. 1730 cm^{-1}.

IR-SPECTRA, measured on a spectrophotometer Perkin Elmer 983G :

Azelaic acid Ergocalciferyl-diester IR	2958	cm^{-1}	ν (CH)
	2930	"	ν (CH)
	2870	"	ν (CH)
	1723	"	ν (C=O) Ester
	1635	"	ν (C=C) of D_2

		1459 cm^{-1}	δ (CH)
		1370 "	δ (CH_3)
		1185 "	ν (C-O) Ester
		973 "	δ (CH) trans
Azelaic acid Retinyl-diester	IR	2982 cm^{-1}	ν (CH)
		2961 "	ν (CH)
		2865 "	ν (CH)
		1728 "	ν (C=O) Ester
		1653 "	ν (C=C)
		1630 "	ν (C=C)
		1605 "	ν (C=C)
		1455 "	δ (CH)
		1377 "	δ (CH_3)
		1360 "	δ (CH_3)
		960 "	δ (CH) of trans (C=C)

Structure of azelaic acid retinyl-diester confirmed by FT Raman-Spectroscopy. The ester-carbonyl-group is not visible.

Sebacic acid Ergocalciferyldiester	IR	2957 cm^{-1}	ν (CH)
		2871 "	ν (CH)
		1723 "	ν (C=O) Ester
		1459 "	δ (CH)
		1371 "	δ (CH_3)
		1183 "	ν (C-O) Ester
		973 "	δ (CH)

3. Preparation of glutaric acid ethyl-Ergocalciferylester

To 400 mg Ergocalciferol (Calciol, Vitamin D_2) in 30 ml toluene at room temperature one adds dropwise 200 mg (excess) glutaric acid-monoethylesterchloride ($bp_{0,07}$ 68-70 °C) and 150 mg N,N-dimethylformamide in 25 ml toluene. After heating for 2 hours to 60 °C, the solvent is distilled off. The residue is chromatographed on a silicagel column with cyclohexane/ethyl acetate (90 : 10) as eluent. One obtains the

Glutaric acid ethyl-Ergocalciferylester RI 1.51656

In a corresponding manner, also other (asymmetric) dicarbonic acid methyl- and ethyldiesters with sterols and vitamin-compounds can be prepared, such as e.g.:

Azelaic acid Ergosteryl-methylester	RI 1.49260
Azelaic acid Cholecalciferyl-methylester	RI 1.49418
Azelaic acid ethyl-Cholecalciferyl-ester	RI 1.52722

Composition examples of spontaneously dispersible agents which contain as substances possessing antitumour activity new dicarbonic acid ester compounds according to the formulae (I) to (VI):

a) 0,5 to 25 % by weight of one or several of the dicarbonic acid ester compounds of the formulae (I) to (VI)
0,1 to 40 % by weight of isopropylmyristate, isopropylpalmitate or Miglyol® 812 (Dynamit Nobel) 20 to 45 % by weight of emulsifier mixture Diphasol® 3873 (CIBA-GEIGY) or the identical product Sermul® EA 188 (SERVO)
20 to 45 % by weight of Invadin® JFC 800 % (CIBA-GEIGY)
0 to 50 % by weight multifunctional glucose derivatives, such as Glucate® SS and Glucamate® SSE-20 (AMERCHOL)

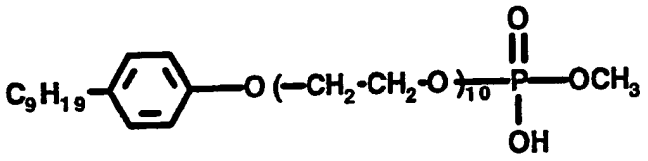
b) 0,5 to 25 % by weight of one or several of the dicarbonic acid ester compounds of the formulae (I) to (VI)
0,1 to 40 % by weight of isopropylmyristate, isopropylpalmitate or Miglyol® 812 (Dynamit Nobel)
20 to 45 % by weight of Invadin® JFC 800 % (CIBA-GEIGY)
20 to 45 % by weight of Soprophor® FL (Rhône-Poulenc)
0 to 50 % by weight multifunctional glucose derivates, such as Glucate® SS and Glucamate® SSE-20 (AMERCHOL)

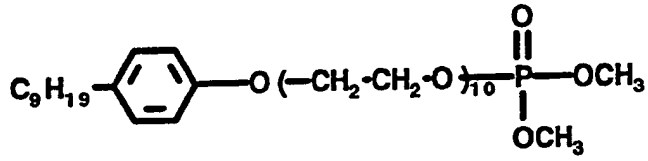
c) 2,5 to 25 % by weight b) 0,5 to 25 % by weight of one or several of the dicarbonic acid ester compounds of the formulae (I) to (VI)
0,1 to 40 % by weight of isopropylmyristate, isopropylpalmitate or Miglyol® 812 (Dynamit Nobel)
20 to 60 % by weight of Invadin® JFC 800 % (CIBA-GEIGY)
20 to 40 % by weight of Amphonyl® CAA and/or CA-AA (Oranienburger Chemikalien A.G., agent: Christ A.G., Aesch/ BL)

Miglyol® 812 is a neutral oil (oleum neutrale) of Dynamit Nobel, which is a triacylglycerol of the coconut fatty acids, the so-called fractionated, middle

chained (C_8 to C_{10}) compounds [i.e. a caprylic/capric triglyceride in the CTFA classification].

Diphasol® 3873 (CIBA-GEIGY) (which is equivalent to SERMUL® EA188) is a surfactant mixture consisting of the following two compounds (50:50) :

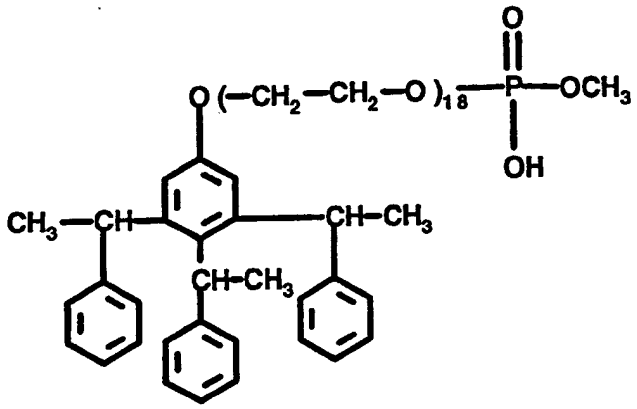




Diphasol® 3873 (CIBA-GEIGY)

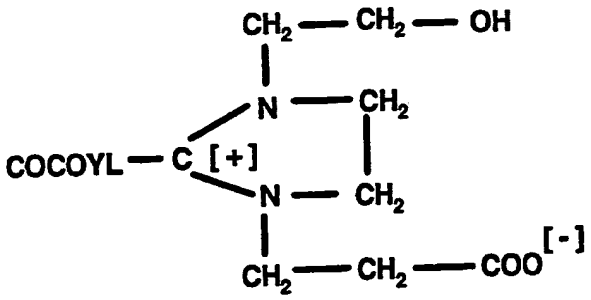
Invadin® JFC 800 % (CIBA-GEIGY) is a tert. octylphenylpolyoxy-ethyleneether with 9 to 10 oxyethylene groups

Soprophor® FL (Rhône-Poulenc) is a tristyrylphenolpolyoxy-ethylene-18-mono/dimethyl-phosphoracidester. with formula:



Soprophor FL (Rhône-Poulenc)

Amphonyl® CAA and CA-AA respectively (Oranienburger Chemikalien A.G.) is an amphoteric, salt and waterfree imidazole derivative of coconut fat, with formula:



Example for the pharmaceutical production of a system's preparation containing the inventive concentrates in the form of "multiple units".

a) Granulation (granules and pellets)

Metolose® 90 SH-4000 (Shin-Etsu Chemical)	90.0 g
Avicel® PH-101	80,3 g
Inventive MARIGENOL®-CONCENTRATE	139,4 g
Aerosil® 200	80,3 g
Σ	390.0 g

Granulation in the high speed mixer or the fluidized bed, with the addition of 110 g ethanol, sieving on a 18 to 42 mesh screen with crushing, drying for 24 h at 40 °C.

b) Enteric and sustained release coating
In the fluidized bed with AQOAT® AS-HG (Shin-Etsu Chemical) and Talc

c) Composition of finished granules or micropellets

Core Material	44 % by weight
Inventive MARIGENOL®-CONCENTRATE	25 % " "
Enteric coating	31 % " "
Σ	100 %

N.B. The pellets or granules according to a) can also be filled without prior coating into capsules which are made of AQOAT® (HPMC-AS-M or HPMC-AS-N), have been sealed with acetone/ethanol 1:1 and can thus perform the functions of pH-control and slow release.

P.S.: MARIGENOL® is a trade-mark (™) of MARIGEN S.A., RIEHEN.

BIOLOGICAL ASSAYS.

The antitumour activity of spontaneously dispersible concentrates containing active substances prepared according to the examples No. 1 to 4 is confirmed by the following test results:

1. In-vitro assays using suitable tumour cell lines

A biological assay system using microtiter plates and serial dilutions has been developed. Batches of 10^4 tumour cells per ml were set up in culture medium RPMI 1640 and inactivated with 10 % of fetal calf serum (GIBCO); they are spread at a density low enough to enable them to grow during the assay, in so-called non-confluent monolayers. Samples are added after 6 - 24 hours, with 100 µl per row, to which 100 µl of medium are added in the first well. Half of this mixture is withdrawn, transferred into the next well and again treated with 100 µl of medium, etc. This results in an n½ geometrical serial dilution.

In the plaque assay, the samples are incubated at 37 °C for 3 to 5 days under 3½ % of CO_2. They are then stained and fixed using 0.1 % crystal violet (Fluka, Buchs) in a solution of 70 % of methanol, 1 % of formaldehyde and 29 % of water. The samples are evaluated under the microscope, magnification 300x. The greatest cytotoxic dilution is determined. The samples can also be evaluated quantitatively by means of scanning and absorption measurement in a spectrophotometer.

EVALUATION OF RESULTS

TUMOR CELL LINE PREPARATION (CONCENTRATE with) EXPOSURE	PY6 polyoma virus transformed 3T3 mouse FIBROBLASTS In dilution active up to 1 : 40 h	PY6 polyoma virus transformed 3T3 mouse FIBROBLASTS In dilution active up to 1 : 64 h
FUMARIC ACID ERGO-STERYL-DIESTER	9,6 Mio.	19,2 Mio.
MALONIC ACID ERGO-STERYL-DIESTER	1,0 Mio.	2,0 Mio.
AZELAIC ACID CHO-LESTERYL-DIESTER	19,2 Mio.	19,2 Mio.
SEBACIC ACID ERGO-STERYL-ESTER	4,8 Mio.	4,8 Mio.
AZELAIC ACID bis-CALCIFERYL-ESTER	9,6 Mio.	9,6 Mio.
GLUTARIC ACID bis-CALCIFERYL-ESTER	2,2 Mio.	4,4 Mio.
AZELAIC ACID RETINYL-DIESTER	2,0 Mio.	4,0 Mio.
AZELAIC ACID CHOLECALCIOL-MONOESTER	1,0 Mio.	2,0 Mio.
SEBACIC ACID CALCIOL-DIESTER	1,0 Mio.	2,0 Mio.
AZELAIC ACID ERGO-STERYL-METHYLESTER	640'000	
AZELAIC ACID CHOLE-CALCIFERYL-METHYL-ESTER	640'000	

CONTINUATION

TUMOR CELL LINE PREPARATION (CONCENTRATE with) EXPOSURE	PY6 Polyoma-Virus-transformed 3T3 Mouse FIBROBLASTS In dilution active up to 1 : 5 days
CROTONIC ACID-D_3-DIESTER AZELAIC ACID-D_3-DIESTER MALONIC ACID-ERGOSTERYL-DIESTER	2'560'000 1'128'000 40'000'000

DOSE-EFFICACY ASSAYS in-vivo (mouse)

The assay was conducted with old reproduction animals Balb-c fem. (Charles River, Calco, Italy)

Tumor-Cell-Line TSA (spontaneous murine mammalian adenocarcinoma), subcutaneously positioned with 4 x 10^4 cells.

The active substance was administered by syringe as aqueous microemulsion 1:100'000 (= 10 ppm active substance content), dose: 0,5 ml/per os 1 times/day, respectively 2 times/day.

Groups of 5 animals each. (This kind of tumor "catches" almost without exception and grows regularly as a subcutaenous mass). This fact allows to keep the statistical classes relatively small.

As a guiding substance the crotonic acid Cholecalciferyl-Ester (C 4:1-D_3) was administered in the form of an inventive concentrate and of the aqueous microemulsion prepared therewith.

Measurement: palpation of the solid tumor mass : Σ [½ length + ½ breadth] in mm

RESULT in-vivo per os

Duration of treatment	Controls	D_3-Crotonate ($C4:1-D_3$)	D_2-Oleate ($C18:1-D_2$)
		1 x 0,5 ml/die	
0 days	0 0 0 0 0	0 0 0 0 0	
35 days	0 0 0 0 2	0 0 0 0 0	
45 days	0 0 0 0 6	0 0 5 5 6	
55 days	0 0 7 14	0 0 7 12 13	
65 days	0 6 20	0 0 15 20 20	
		2 x 0,5 ml/die	2 x 0,5 ml/die
0 days	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
35 days	0 0 0 0 2	0 0 0 0 0	0 0 0 0 0
45 days	0 0 0 0 6	0 0 0 0 0	0 0 0
55 days	0 0 7 14	0 0 0 0 0	0 0 0
65 days	0 6 20	0 0 0 0 0	2 3 8

Assays made under the guidance of Prof. Dott. Guido FORNI, Università degli Studi di TORINO, Scuola di Medicina, Istituto di Microbiologia.
The adenocarcinoma TSA is a very aggressive tumor line. It is being used as standard for evaluating cytostatica.

Action pattern of MARIGENOL®-concentrates in topical administration
In order to gain first insights into the action pattern of the inventive concentrates in *topical administration* tests were conducted with the naked mouse, using as key substances: Ergosteryl-Undecenoate and Ergosteryl-Crotonate (Topi nu/nu f., Charles River, Calco, Italy), which were immuno-depressed by means of splenectomy and subletal irradiation 4,5 Gy Cs^{137}, dose 0,5 Gy/min.

The human lung adenocarcinoma ADK-P (an extremely malignant cell line) was implanted 3 days later, subcutaneously into the flank with 10^6 cells. This sort of tumor is very reliable, it "catches" securely and without exception. It grows regularly and forms a solid mass und the skin. Period of latency 10 ± 2 days, subsequently growth of the solid mass from 3 to 10 mm within a period

of 61 ± 5 days. Close observation revealed no detectable metastasis, no spontaneous recidivism. Killing of the animals when the tumor mass reaches 15 mm.

Injection of a microemulsion 1:100'000 containing 10 ppm active substance when the tumor mass reached 4 mm of size, with 1 ml microemulsion 2 - 3 times per day *directly into the tumor mass and its surrounding.*

RESULTS: Retardation of the tumor growth by ca. 15 days. ONE COMPLETE REGRESSION BY FOUR, after 20 days of treatment. Observation of the treated animals lin the ensuign period during further 90 days. There were no metastases, no relaps. Pathological control of organs normal. No neoplastic tissues formed.

This may allow the conclusion that the described results could be achieved without the immediate participation of or the mediation by the immune system.

FORMATION OF ANTIBODIES

Tests were conducted on behalf of MARIGEN by Readysysteme A.G., Bad Zurzach/Gamma, University of Liège, Belgium, on rabbits, with the objective of ascertaining a possible *reaction of the immune system* when adminis-tering inventive sterolester compounds, in particular:

CALCIOL-LINOLEATE [C 18:2-D_2-Ester]

as key substance, the coemulgator C 11:1-Citronellylester and the "combination" sterolester/terpenester 25/75. Analysis of the serum using the ELISA-method did not evidence any traces of antibodies in the form of immunoglobulines. There were no allergens becoming manifest.

BLOOD VALUES

The surface tension was measured using human peripheral blood immediately after drafting and mixing with an inventive concentrate, from which a microemulsion was prepared by adding distilled water, as described above.

BLOOD VALUES PROBES	FULL BLOOD EDTA mNm^{-1}		FULL BLOOD Na-Citricum mNm^{-1}	
	Plate	Ring	Plate	Ring
1) Full blood (whole blood)	51,6	51,6	51,4	51,4
2) Full blood mixed 1 : 0,5 with Azelaic acid Ergosteryl-di-ester-concentrate 1:1'000 (C) = 100 ppm active substance content	47,9	45,7		
3) Full blood mixed 1 : 1 with Azelaic acid Ergosteryl-di-ester-concentrate 1:1'000 (C)	45,4	45,4	44,8	44,1
4) Full blood mixed 1 : 5 with Azelaic acid Ergosteryl-di-ester-concentrate 1:1'000 (C)		44,9	46,4	45,2
5) Full blood mixed 1 : 5 with Ergosteryl-Undecenoate-concentrate 1:1'000 (C)		38,3	39,6	39,8
6) Full blood mixed 1 : 1 with Cholecalciferyl-Undecenoate concentrate 1 : 100 (C) = 1'000 ppm active substance content !		32,1	32,7	32,9

N.B.: Measurements conducted on a Krüss Digital-Tensiometer K10T
Vacutainer Becton Dickinson

The surface tension of normal blood amounts to 56,2 mNm^{-1} (Average). Cf-Geigy Scientific Tables Volume 3, Physical Chemistry, Composition of Blood, Hematology, Somatometric Data, 8th ed., Basel, 1984, p. 69.

METABOLISM

During 5 consecutive days, healthy persons took 2 capsules/day each with 600 mg of an inventive concentrate having a 10%-STEROLESTER CONTENT. (Concretely: RETINOIC ACID ERGOSTERYL-ESTER as analytically suitable

guiding substance). By means of thin layer chromatography, it could be shown that:

- a part of the sterolester was eliminated in the urine, i.e. in the *ester form*
- a part of the sterolester could be traced, equally unchanged, in the blood
- the blood status evidenced no changes whatsoever, which gives an important indication regarding the toleration of the micellar solubilization and transport system used.

It may be emphasized that the diffusion of the inventive concentrates and microemulsions obeys a *general physical principle, which was taken as guideline when optimizing the composition of these inventive concentrates.* The penetration of the plasma membrane of tumor cells (and the subsequent spreading in the cell plasma) is illustrated by means of EM-photographs, using as key substances a 2%-concentrate prepared with ERGOSTEROL-10-UNDECENOATE and ERGOSTEROL-all trans-RETINATE respectively. Cf. Figures 1/4 and 2/4 in the Annexure.

The *analytical proof of the penetration of the plasma membrane* of the tumor cell can be furnished using as method the "Micellar Electrokinetic Capillary Chromatography - MECC." This requires the preparation of an appropriate buffer system and the careful definition of the conditions for the assess-ment of the electroosmotic flux. In both samples, the concentrated urine and the blood fraction, the sterolesters could clearly and unequivocally be traced. Cf. the figures 3/4 and 4/4 in the annexure.

Figure 1/4 demonstrates the penetration of the membrane of Py6-cells (Fibroblasts) with ERGOSTEROL-10-UNDECENOATE

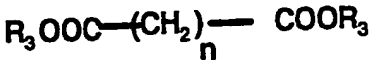
Figure 2/4 demonstrates the penetration of the membrane of Py6-cells (Fibroblasts) with ERGOSTEROL-all trans-RETINATE

Figure 3/4 gives as reference the MECC-diagram of the control substance ERGOSTEROL-all trans-RETINATE

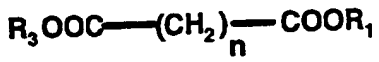
Figure 4/4 shows the MECC-diagrams for the blood probe and the urine probe containing ERGOSTEROL-all trans-RETINATE.

PATENT CLAIMS

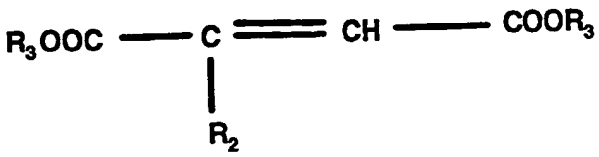
1) Dicarbonic acid esters of the formulae (I) to (VI)



(I)



(II)



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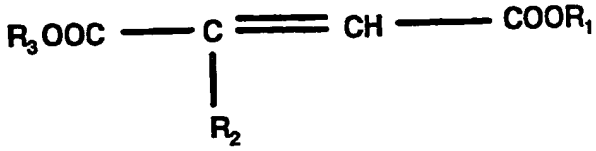
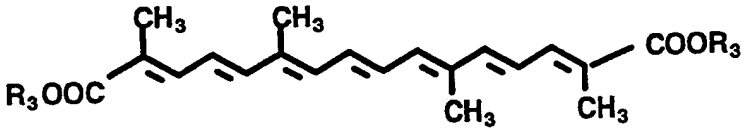
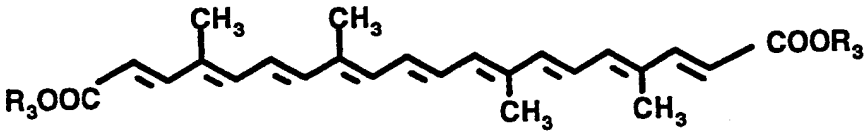


(IV)

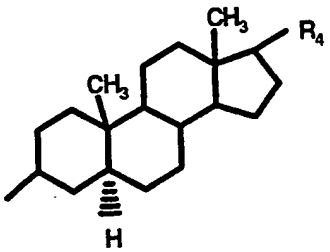


(V)

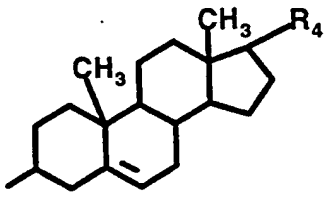


(VI)

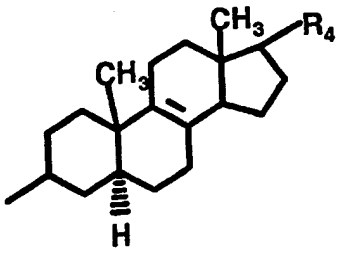
whereby in the formulae (I) to (II) the letter n stands for the numbers 1 to 18, and in the formulae (I) to (VI) R_1 designates a C_1 to C_{20} alkyl or C_2 to C_{20} alkenyl group, R_2 means hydrogen or methyl and R_3 represents one of the radicals of the following formulae:



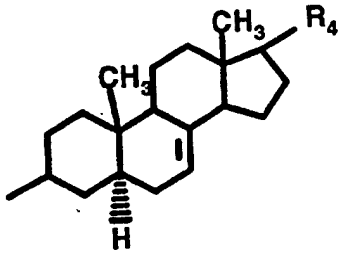
(VII)



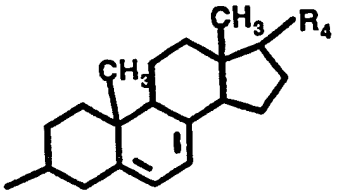
(VIII)



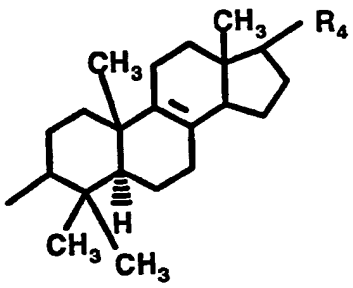
(IX)



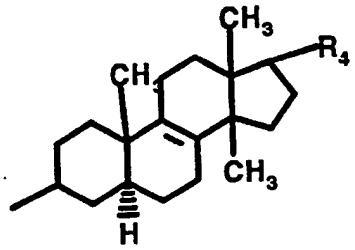
(X)



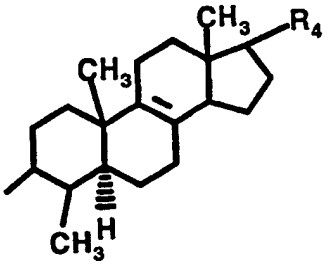
(XI)



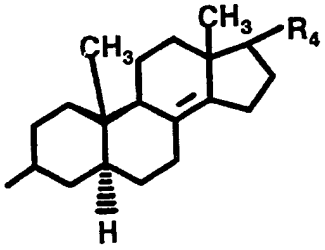
(XII)



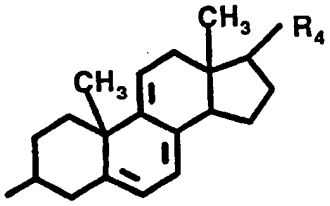
(XIII)



(XIV)

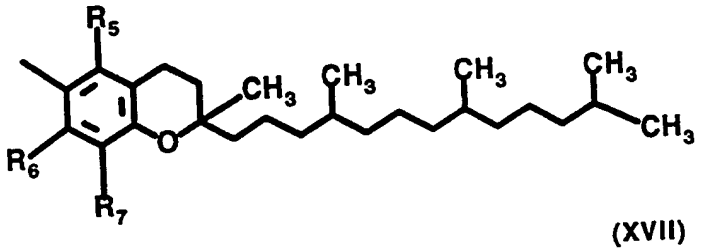


(XV)

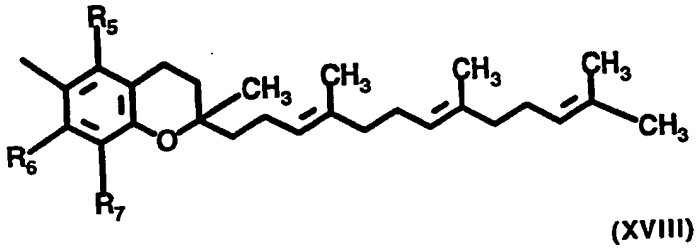


(XVI)

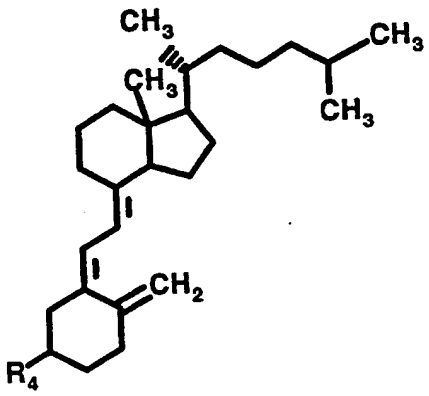
whereby in the formulae (VII) to (XVI) the radical R_4 stands for a C_1 to C_{10} alkyl or a C_2 to C_{10} alkenyl group,



(XVII)



(XVIII)



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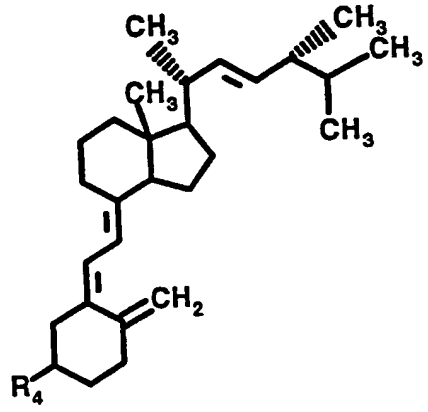

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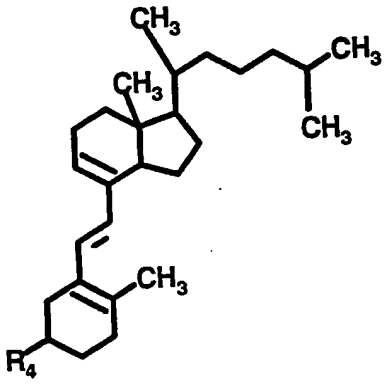

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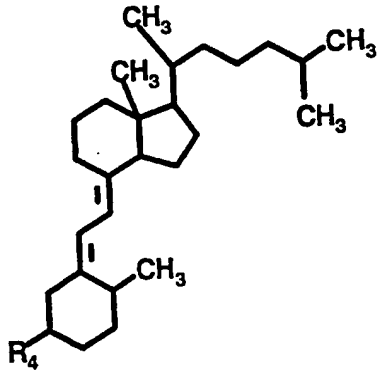
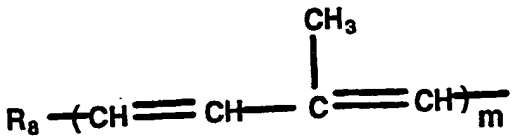
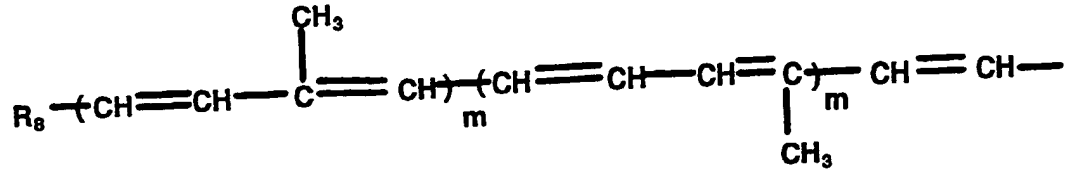


(VI)

and where in the formulae (XVII) and (XVIII) the radicals R_5, R_6 und R_7 represent hydrogen or methyl,

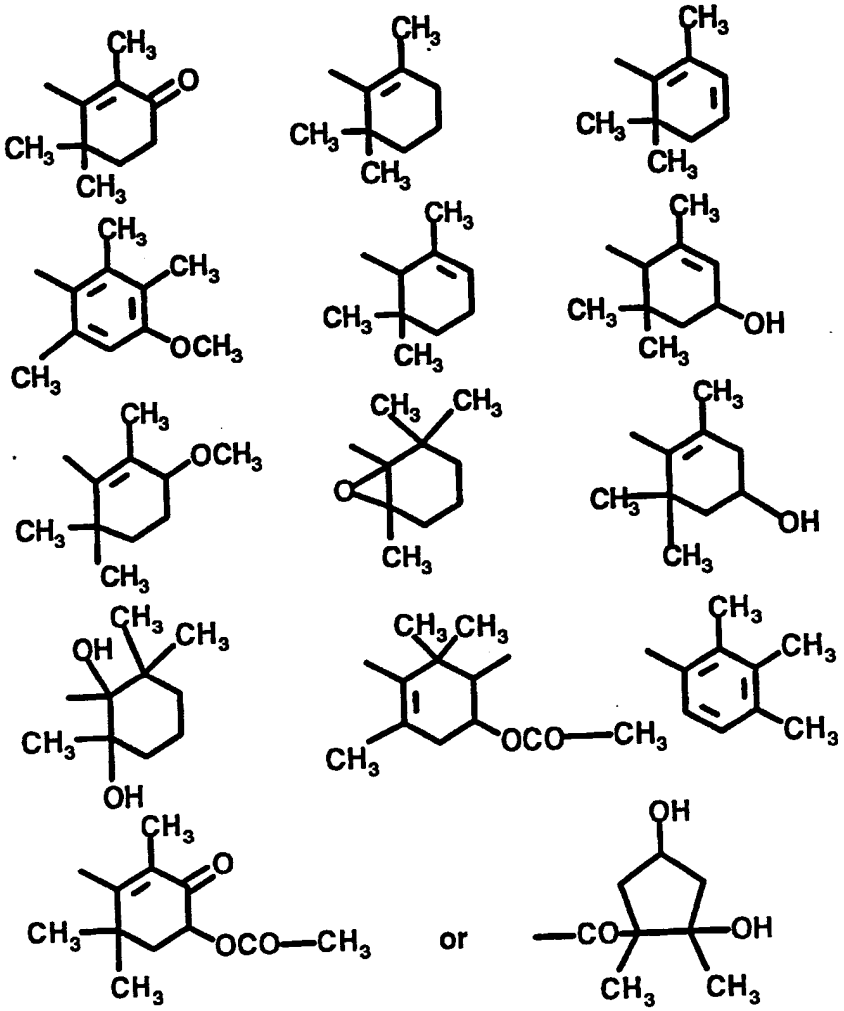


(XXIII)

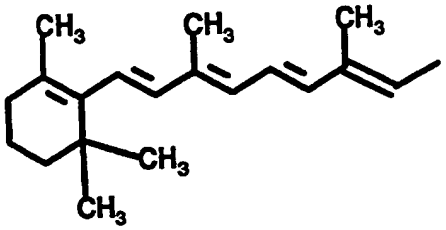


(XXIV)

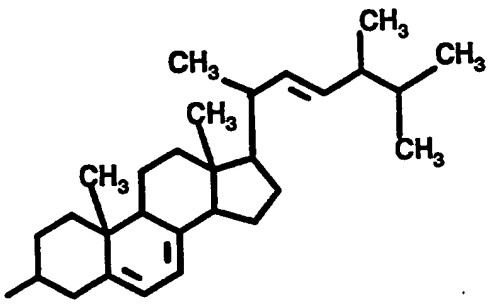
whereby in the formulae (XXIII) and (XXIV) the letter m means the numbers 1, 2, 3, 4 or 5 and R_8 stands for one of the radicals of formulae:



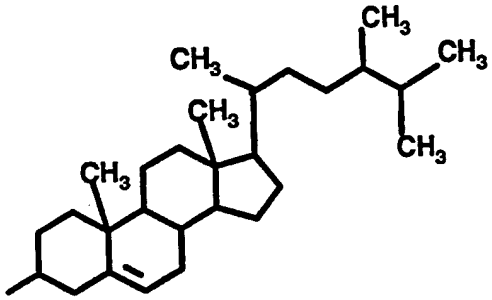
2. The compounds according to claim 1, whereby in the formulae (I) and (II) the letter n means the number 1 to 8, R_1 stands for methyl or ethyl, R_2 is hydrogen and R_3 determines one of the radicals of formulae (XXV) to (XXX)



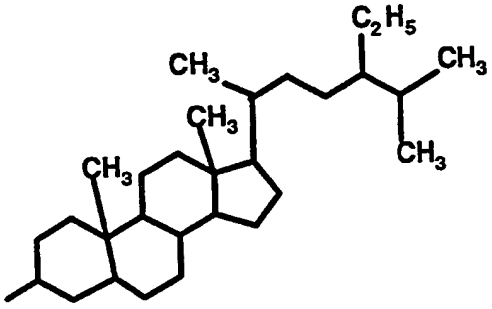
(XXV)



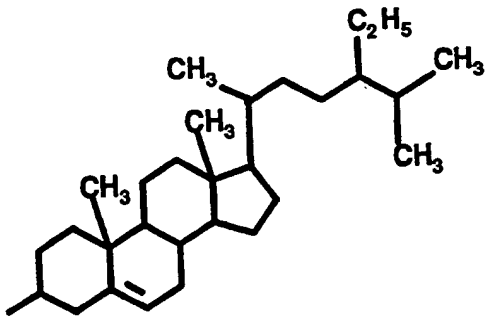
(XXVI)



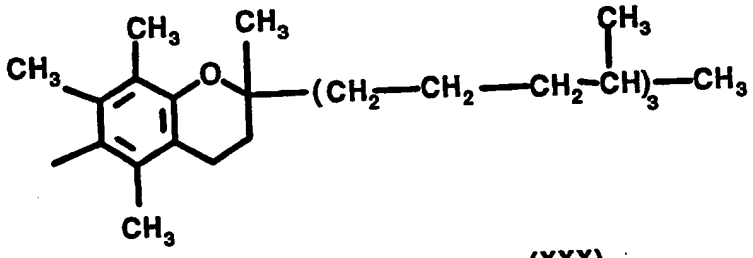
(XXVII)



(XXVIII)



(XXIX)



(XXX)

3. The compounds according to claim 2

Malonic acid bis-Stigmasterylester
Succinic acid bis-Stigmasterylester
Glutaric acid bis-Stigmasterylester
Adipic acid bis-Stigmasterylester
Pimelic acid bis-Stigmasterylester
Suberic acid bis-Stigmasterylester
Azelaic acid bis-Stigmasterylester
Sebacic acid bis-Stigmasterylester

Azelaic acid bis-b-Sitosterylester
Sebacic acid bis-b-Sitosterylester
Azelaic acid bis-Ergosterylester
Sebacic acid bis-Ergosterylester
Azelaic acid bis-Cholesterylester
Sebacic acid bis-Cholesterylester

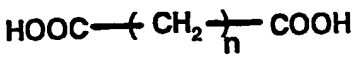
Maleic acid bis-Stigmasterylester
Fumaric acid bis-Stigmasterylester
Maleic acid bis-β-Sitosterylester
Fumaric acid bis-β-Sitosterylester
Maleic acid bis-Ergosterylester

Fumaric acid bis-Ergosterylester
Maleic acid bis-Cholesterylester
Fumaric acid bis-Cholesterylester

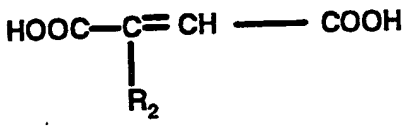
Azelaic acid Calciferyl-diester
Sebacic acid Calciferyl-diester
Azelaic acid Cholecalciferyl-diester
Sebacic acid Cholecalciferyl-diester
Azelaic acid DL-α-Tocopheryl-diester
Sebacic acid DL-α-Tocopheryl-diester

Glutaric acid ethyl-Calciferylester
Glutaric acid Calciferyl-methylester
Fumaric acid ethyl-Calciferylester
Fumaric acid Calciferyl-methylester
Azelaic acid ethyl-Calciferylester
Azelaic acid Calciferyl-methylester
Azelaic acid ethyl-Cholecalciferylester
Azelaic acid Cholecalciferyl-methylester
Sebacic acid ethyl-Calciferylester
Sebacic acid Calciferyl-methylester
Sebacic acid ethyl-Cholecalciferylester
Sebacic acid Cholecalciferyl-methylester

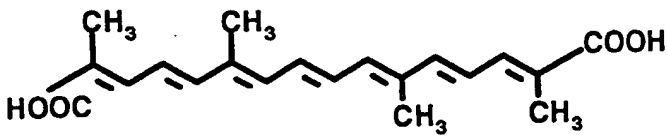
4. Procedure for the preparation of compounds described in the formulae (I), (III), (V) and (VI) according to claim 1, characterized by reacting a compound of the formulae (XXXI) to (XXXIV)



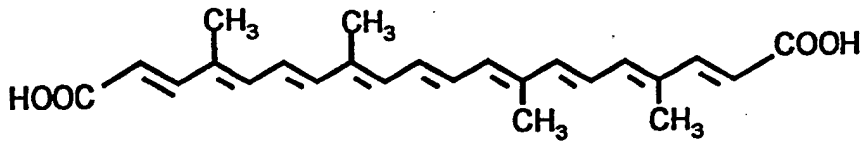
(XXXI)



(XXXII)



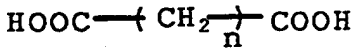
(XXXIII)



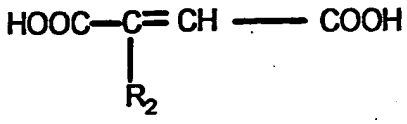
(XXXIV)

in which the letter n represents the numbers 1 to 18 and R_2 means hydrogen or methyl, with 1,1'-carbonyldiimidazole at 25 to 70 °C under addition of a catalytic amount of an alcoholate in an indifferent solvent, such as tetrahydrofuran, benzene, toluene or chloroform, followed by alcoholysis of the imidazolides formed with a sterol, a vitamin-D or a vitamin-E compound.

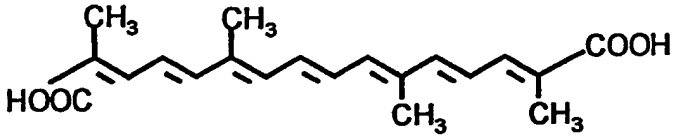
5. Procedure for the preparation of compounds described in the formulae (I), (III), (V) and (VI) according to claim 1, characterized by reacting a compound of the formulae (XXXI) to (XXXIV)



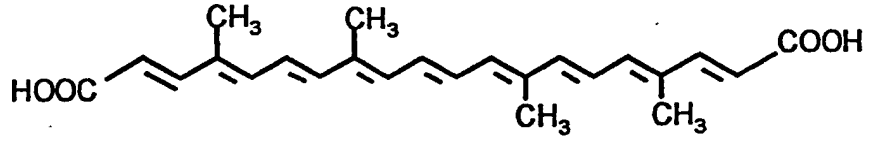
(XXXI)



(XXXII)



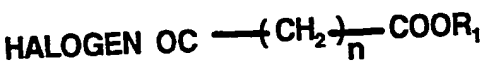
(XXXIII)



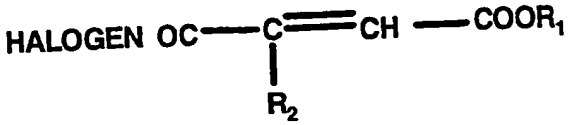
(XXXIV)

in which the letter n means the number 1 to 18 and R_2 stands for hydrogen or methyl, with a chlorination or bromination agent in oder to prepare the dichloride or the dibromide, followed by the reaction of the dichloride or dibromide formed with a sterol, a vitamin-D or a vitamin-E compound at a temperature of between 40 and 80 °C in an indifferent solvent.

6. Procedure for the preparation of compounds of formulae (II) and (IV) according to claim 1, characterized by reacting a compound of formulae (XXXV) or (XXXVI)



(XXXV)



(XXXVI)

in which the letter n determines the numbers 1 to 18, R_1 stands for alkyl or alkenyl and R_2 means hydrogen or methyl, with a sterol, a vitamin-D or a vitamin-E compound at a temperature of between 40 and 80 °C in an indifferent solvent and in the presence of a catalyst.

7. Spontaneously dispersible concentrate which contains as the antitumor and biotenside components
0.001 to 25 % by weight of individual esters with dicarbonic acids according to formulae (I) to (VI), and combinations of such compounds respectively
0.001 to 40 % by weight of a solvent or solvent mixture which is pharmaceutically acceptable and acts as a hydrotropic or coemulsifier
0.001 to 90 % by weight of a pharmaceutically acceptable surfactant or surfactant mixture, and optionally
up to 10 % by weight of a vitamin or provitamin
up to 10 % by weight of a free fatty acid, and if appropriate, customary excipients and/or diluents.

8. A pharmaceutical composition comprising 1 to 95 % of the spontaneously dispersible concentrate according to claim 1, and which further comprises an amount up to 10 % by weight of a pharmaceutically acceptable excipient, diluent, stabilizer, or a combination thereof.

9. A therapeutic system's preparation according to claim 8, which contains 44 parts of core material for granules or pellets, 25 parts of the spontaneously dispersible concentrate and 31 parts of enteric and slow-release-coating on the basis of hydroxylpropyl-methylcellulose-acetate-succinate.

10. A therapeutic system's preparation according to claim 8, which contains 64 parts of core material for granules and pellets respectively, and 36 parts of the inventive concentrate and which is filled into capsules made from hydroxypropyl-methyl-cellulose-acetate-succinate.